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(54) Title: METHODS FOR OBTAINING PLANT VARIETIES

## (57) Abstract

An isolated and purified DNA molecule comprising a polynucleotide sequence encoding a polypeptide functionally involved in the DNA mismatch repair system of a plant.

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## Methods for Obtaining Plant Varieties

### TECHNICAL FIELD

The present invention relates to nucleotide sequences which encode polypeptides involved in the DNA mismatch repair systems of plants, and to the polypeptides encoded by those nucleotide sequences. The invention also relates to nucleotide sequences and polypeptide sequences for use in altering the DNA mismatch repair system in plants. The invention also relates to a process for altering the DNA mismatch repair system of a plant cell, to a process for increasing genetic variations in plants and to processes for obtaining plants having a desired characteristic.

10

### BACKGROUND OF THE INVENTION

Plant breeding essentially relies on and makes use of genetic variation which occurs naturally within and between members of a family, a genus, a species or a subspecies. Another source of genetic variation is the introduction of genes from other organisms which may or may not be related to the host plant.

15 Allelic loci or non-allelic genes which constitute or contribute to desired quantitative (e.g. growth performance, yield, etc.) or qualitative (e.g. deposition, content and composition of seed storage products; pathogen resistance genes; etc.) traits that are absent, incomplete or inefficient in a species or subspecies of interest are typically introduced by the plant breeder from other species or subspecies, or *de novo*. This  
20 introduction is often done by crossing, provided that the species to be crossed are sexually compatible. Other means of introducing genomes, individual chromosomes or genes into plant cells or plants are well known in the art. They include cell fusion, chemically aided transfection (Schocher et al., 1986, Biotechnology 4: 1093) and ballistic (McCabe et al., 1988, Biotechnology 6: 923), microinjection (Neuhaus et al., 1987, TAG 75: 30),  
25 electroporation of protoplasts (Chupeau et al., 1989, Biotechnology 7: 53) or microbial transformation methods such as Agrobacterium mediated transformation (Horsch et al., 1985, Science 227: 1229; Hiei et al., 1996, Biotechnology 14: 745).

However, when a foreign genome, chromosome or gene is introduced into a plant, it will often segregate in subsequent generations from the genome of the recipient plant or  
30 plant cell during mitotic and meiotic cell divisions and, in consequence, become lost from the host plant or plant cell into which it had been introduced. Occasionally, however, the introduced genome, chromosome or gene physically combines entirely or in part with the genome, chromosome or gene of the host plant or plant cell in a process which is called recombination.

35 Recombination involves the exchange of covalent linkages between DNA molecules in regions of identical or similar sequence. It is referred to here as homologous recombination if donor and recipient DNA are identical or nearly identical (at least 99%

base sequence identity), and as homeologous recombination if donor and recipient DNA are not identical but are similar (less than 99% base sequence identity).

The ability of two genomes, chromosomes or genes to recombine is known to depend largely on the evolutionary relation between them and thus on the degree of 5 sequence similarity between the two DNA molecules. Whereas homologous recombination is frequently observed during mitosis and meiosis, homeologous recombination is rarely or never seen.

From a breeder's perspective, the limits within which homologous recombination occurs, therefore, define a genetic barrier between species, varieties or lines, in contrast 10 to homeologous recombination which can break this barrier. Homeologous recombination is thus of great importance for plant breeding. Accordingly there is a need for a process for enhancing the frequency of homeologous recombination in plants. In particular, there is a need for a process of increasing homeologous recombination to significantly shorten 15 the length of breeding programs by reducing the number of crosses required to obtain an otherwise rare recombination event.

At least in *Escherichia coli*, homologous and homeologous recombination are known to share a common pathway that requires among others the proteins RecA, RecB, RecC, RecD and makes use of the SOS induced RuvA and RuvB, respectively. It has been suggested that mating induced recombination follows the Double-Strand Break Repair 20 model (Szostak et al., 1983, Cell 33, 25-35), which is widely used to describe genetic recombination in eukaryotes. Following the alignment of homologous or homeologous DNA double helices the RecA protein mediates an exchange of a single DNA strand from the donor helix to the aligned recipient DNA helix. The incoming strand screens the recipient helix for sequence complementarity, seeking to form a heteroduplex by hydrogen 25 bonding the complementary strand. The displaced homologous or homeologous strand of the recipient helix is guided into the donor helix where it base pairs with its counterpart strand to form a second heteroduplex. The resulting branch point then migrates along the aligned chromosomes thereby elongating and thus stabilising the initial heteroduplexes. Single stranded gaps (if present) are closed by DNA synthesis. The strand cross overs 30 (Holliday junction) are eventually resolved enzymatically to yield the recombination products.

Although in wild type *E. coli* homologous and homeologous recombination are thus mechanistically similar if not identical, homologous recombination in conjugational crosses *E. coli* x *E. coli* occurs five orders of magnitude more frequently than 35 homeologous recombination in conjugational crosses *E. coli* x *S. typhimurium* (Matic et al. 1995; Cell 80, 507-515). The imbalance in favour of homologous recombination was shown to be caused largely by the bacterial MisMatch Repair (MMR) system since its

inactivation increased the frequency of homeologous recombination in *E. coli* up to 1000 fold (Rayssiguier et al. 1989, Nature 342, 396-401).

In *E. coli*, the MMR system (reviewed by Modrich 1991, Annual Rev Genetics 25, 229-253) is composed of only three proteins known as MutS, MutL and MutH. MutS 5 recognizes and binds to base pair mismatches. MutL then forms a stable complex with mismatch bound MutS. This protein complex now activates the MutH intrinsic single stranded endonuclease which nicks the strand containing the misplaced base and thereby prepares the template for DNA repair enzymes.

During recombination, MMR components inhibit homeologous recombination. In 10 vitro experiments demonstrated that MutS in complex with MutL binds to mismatches at the recombination branch point and physically blocks RecA mediated strand exchange and heteroduplex formation (Worth et al., 1994; PNAS 91, 3238-3241). Interestingly, the SOS dependent RuvAB mediated branch migration is insensitive to MutS/MutL, explaining the observed slight increase in SOS dependent homeologous recombination. 15 Homeologous mating even induces the SOS response, thereby taking advantage of RuvAB induction (Matic et al. 1995, Cell 80, 507-515).

The MMR system thus appears to be a genetic guardian over genome stability in *E. coli*. In this role it essentially determines the extent to which genetic isolation, that is, speciation, occurs. The diminished sensitivity of the SOS system to MMR, however, 20 allows (within limits) for rapid genomic changes at times of stress, providing the means for fast adaptation to altered environmental conditions and thus contributing to intraspecies genetic variation and species evolution.

The important role of MMR in preserving genomic integrity has been established also in certain eukaryotes. In its efficiency, the human MMR, for example, may even 25 counteract potential gene therapy tools such as triple-helix forming oligonucleotides including RNA-DNA hybrid molecules (Havre et al., 1993, J. Virology 67: 7234-7331; Wang et al., 1995, Mol. Cell. Biol. 15: 1759-1768; Kotani et al., 1996, Mol. Gen. Genetics 250: 626-634; Cole-Strauss et al., 1996, Science 273: 1387-1389). Such oligonucleotides are designed to introduce single base changes into selected DNA target 30 sequences in order to inactivate for example cancer genes or to restore their normal function. The resulting base mismatches however are recognised by the mismatch repair system which then directs removal of the mismatched base, thereby reducing the efficiency of oligonucleotide induced site-specific mutagenesis.

To date, two families of related genes, homologous to the bacterial MutS and MutL 35 genes have been identified or isolated in yeast and mammals (recent reviews by Arnheim and Shibata, 1997, Curr. Opinion Genet. Dev. 7, 364-370; Modrich and Lahue, 1996, Annual Rev. Biochem. 65, 101-133; Umar and Kunkel, 1996, Eur. J. Biochem. 238, 297-307). Biochemical and genetic analysis indicated that eukaryotic MutS homologs (MSH)

and MutL homologs (MLH, PMS), respectively, fulfil similar protein functions as their bacterial counterparts. Their relative abundance, however, could reflect different mismatch specificity and/or specialisation for different tissues or organelles or developmental processes such as mitotic versus meiotic recombination.

To date, six different genes homologous to *MutS* have been isolated in yeast (*yMSH*), and their homologs have been found in mouse (*mMSH*) and human (*hMSH*), respectively. Encoded proteins *yMSH2*, *yMSH3* and *yMSH6* appear to be the main *MutS* homologs involved in MMR during mitosis and meiosis in yeast, where the complementary proteins *MSH3* and *MSH6* alternatively associate with *MSH2* to recognise different mismatch substrates (Masischky et al., 1996, *Genes Dev.* 10, 407-420). Similar protein interactions have been demonstrated for the human homologs *hMSH2*, *hMSH3* and *hMSH6* (Acharya et al., 1996, *PNAS* 93, 13629-13634).

*MutL* homologs (MLH and PMS), recently reviewed by Modrich and Lahue (1996, *Annual Rev. Biochem.* 65, 101-133) have so far been found in yeast (*yMLH1* and *yPMS1*), mouse (*mPMS2*) and human (*hMLH1*, *hPMS1* and *hPMS2*). The *hPMS2* is a member of a family of at least 7 genes (Horii et al., 1994, *Biochem. Biophys. Res. Commun.* 204, 1257-1264) and its gene product is most closely related to *yPMS1*. Prolla et al. (1994, *Science* 265, 1091-1093) presented evidence for *yPMS1* and *yMLH1* to physically associate with each other and, together, to interact with the *MutS* homolog *yMSH2* to form a ternary complex involved in mismatch substrate binding.

However, while medical interest in mismatch repair has prompted extensive research on MMR in bacteria, yeast and mammals, MMR genes have not been isolated from higher plants prior to the present invention and no attempts to adjust the plant MMR to plant breeding needs have been reported.

25

## SUMMARY OF THE INVENTION

According to a first embodiment of the invention, there is provided an isolated and purified DNA molecule comprising a polynucleotide sequence encoding a polypeptide functionally involved in the DNA mismatch repair system of a plant. In one form of this embodiment, the invention provides an isolated and purified DNA molecule comprising a polynucleotide sequence encoding a polypeptide which is homologous to a mismatch repair polypeptide of a yeast or of a human. More particularly, the invention provides polynucleotide sequences encoding polypeptides which are homologous to the mismatch repair polypeptides *MSH3* and *MSH6* of *Saccharomyces cerevisiae*. Still more particularly, the invention provides the coding sequences of the genes *AtMSH3* and *AtMSH6* of *Arabidopsis thaliana*, as defined hereinbelow, and polynucleotide sequences encoding polypeptides which are homologous to polypeptides encoded by *AtMSH3* and *AtMSH6*.

According to a second embodiment of the invention, there is provided an isolated and purified polypeptide functionally involved in the DNA mismatch repair system of a plant, for example a polypeptide which is homologous to a mismatch repair polypeptide of a yeast or of a human such as a polypeptide encoded by the genes *AtMSH3* or *AtMSH6* of 5 *Arabidopsis thaliana*, as defined hereinbelow.

According to a third embodiment of the invention, there is provided an isolated and purified DNA molecule comprising a polynucleotide sequence selected from the group consisting of (i) a sequence encoding a polynucleotide which is capable of interfering with the expression of a plant polynucleotide sequence encoding a polypeptide which is 10 homologous to a mismatch repair polypeptide of a yeast or of a human and thereby disabling said plant polynucleotide sequence; and (ii) a sequence encoding a polypeptide capable of disrupting the DNA mismatch repair system of a plant.

According to a fourth embodiment of the invention there is provided a chimeric gene comprising a DNA sequence selected from the group consisting of (i) a sequence encoding 15 a polynucleotide which is capable of interfering with the expression of a plant polynucleotide sequence encoding a polypeptide which is homologous to a mismatch repair polypeptide of a yeast or of a human and thereby disabling said plant polynucleotide sequence, and (ii) a sequence encoding a polypeptide capable of disrupting the DNA mismatch repair system of a plant: together with at least one regulation element capable of 20 functioning in a plant cell. Examples of such regulation elements include constitutive, inducible, tissue type specific and cell type specific promoters such as 35S, NOS, PR1a, AoPR1 and DMC1. Typically, a chimeric gene of the fourth embodiment will also include at least one terminator sequence, more typically exactly one terminator sequence.

In the third and fourth embodiments, said interference, by said polynucleotide 25 sequence, with the expression of a plant polynucleotide sequence encoding a polypeptide which is homologous to a mismatch repair peptide of a yeast or a human typically occurs by hybridisation or by co-suppression.

According to a fifth embodiment of the invention there is provided a plasmid or vector comprising a chimeric gene of the fourth embodiment. A vector of the fifth 30 embodiment may be, for example, a viral vector or a bacterial vector.

According to a sixth embodiment of the invention, there is provided a plant cell stably transformed, transfected or electroporated with a plasmid or vector of the fifth embodiment.

According to seventh embodiment of the invention, there is provided a plant 35 comprising a cell of the sixth embodiment.

According to an eighth embodiment of the invention, there is provided a process for at least partially inactivating a DNA mismatch repair system of a plant cell, comprising

transforming or transfecting said plant cell with a DNA sequence of the third embodiment or a chimeric gene of the fourth embodiment or a plasmid or vector of the fifth embodiment, and causing said DNA sequence to express said polynucleotide or said polypeptide.

5 According to a ninth embodiment of the invention, there is provided a process for increasing genetic variation in a plant comprising obtaining a hybrid plant from a first plant and a second plant, or cells thereof, said first and second plants being genetically different; altering the mismatch repair system in said hybrid plant; permitting said hybrid plant to self-fertilise and produce offspring plants; and screening said offspring plants for  
10 plants in which homeologous recombination has occurred. For example, homeologous recombination may be evidenced by new genetic linkage of a desired characteristic trait or of a gene which contributes to a desired characteristic trait.

According to a tenth embodiment of the invention there is provided a process for obtaining a plant having a desired characteristic, comprising altering the mismatch repair  
15 system in a plant, cell or plurality of cells of a plant which does not have said desired characteristic, permitting mutations to persist in said cells to produce mutated plant cells, deriving plants from said mutated plant cells, and screening said plants for a plant having said desired characteristic.

In a preferred form of the ninth and tenth embodiments of the invention, the step of  
20 altering the mismatch repair system comprises introducing into said hybrid plant, plant, cell or cells a chimeric gene of the fourth embodiment and permitting the chimeric gene to express a polynucleotide which is capable of interfering with the expression of a plant polynucleotide sequence in a mismatch repair gene of the hybrid plant, plant, cell or cells, or a polypeptide capable of disrupting the DNA mismatch repair system of the hybrid  
25 plant or cells.

In other embodiments, the invention provides (a) an oligonucleotide capable of hybridising at 45°C under standard PCR conditions to a DNA molecule of the first embodiment; (b) an oligonucleotide capable of hybridising at 45°C under standard PCR conditions to the DNA of SEQ ID NO: 18 and (c) an oligonucleotide capable of  
30 hybridising at 45°C under standard PCR conditions to the DNA of SEQ ID NO:30; with the proviso that the oligonucleotide of (a), (b) and (c) is other than SEQ ID NO:1 or SEQ ID NO:2.

#### BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 provides a diagrammatic representation of the primer sequences used to  
35 isolate *AtMSH3*.

Figure 2 is a plasmid map of clone 52, showing restriction enzyme cleavage sites in the 5' half of the full-length cDNA for *AtMSH3*.

Figure 3 is a plasmid map of clone 13, showing restriction enzyme cleavage sites in the 3' half of the full-length cDNA for *AtMSH3*.

Figure 4 is a sequence listing of the coding sequence of *AtMSH3*, together with a deduced sequence of the encoded polypeptide.

5 Figure 5 is a protein alignment of yeast (*Saccharomyces cerevisiae*) and *Arabidopsis thaliana* MSH3 protein.

Figure 6 provides a diagrammatic representation of the primer sequences used to isolate *AtMSH6*.

Figure 7 is a plasmid map of clone 43, showing restriction enzyme cleavage sites in 10 the 5' half of the full-length cDNA for *AtMSH6*.

Figure 8 is a plasmid map of clone 62, showing restriction enzyme cleavage sites in the 3' half of the full-length cDNA for *AtMSH6*.

Figure 9 is a sequence listing of the coding sequence of *AtMSH6*, together with a deduced sequence of the encoded polypeptide.

15 Figure 10 is a protein alignment of yeast (*Saccharomyces cerevisiae*) and *Arabidopsis thaliana* MSH6 protein.

Figure 11 is a genomic sequence listing of *AtMSH6*.

Figure 12 is a plasmid map of plasmid pPF13.

Figure 13 is a plasmid map of plasmid pPF14.

20 Figure 14 is a plasmid map of plasmid pCW186.

Figure 15 is a plasmid map of plasmid pCW187.

Figure 16 is a plasmid map of plasmid pPF66.

Figure 17 is a plasmid map of plasmid pPF57.

Figure 18 is a diagrammatic representation of an antisense gene construction for use 25 in homeologous meiotic recombination.

Figure 19 is a plasmid map of plasmid p3243.

## DETAILED DESCRIPTION OF THE INVENTION

The present invention is based on the inventors' discovery that there exist in higher plants genes which are homologous to MMR genes in *E. coli*, and to MMR genes in 30 yeasts and humans.

Thus, the inventors have identified genes, herein designated *AtMSH3* and *AtMSH6*, of the plant *Arabidopsis thaliana* which encode the proteins AtMSH3 and AtMSH6. These plant proteins are homologous to yMSH3 and yMSH6, respectively. The present inventors have isolated cDNAs encoding the proteins AtMSH3 and AtMSH6 and have 35 isolated the complete gene encoding AtMSH6. Given the teaching herein, other genes (for example AtMSH2, and genes of other plants) may be obtained which are involved in DNA mismatch repair in plants, including other genes which encode polypeptides homologous to MMR proteins of yeasts or humans, such as genes which encode

polypeptides homologous to yeast MSH2, MLH1 or PMS2, or to human MLH1, PMS1 or PMS2. For example, given the teaching herein, genes of members of the *Brassicaceae* family or of other unrelated families, for example the *Poaceae*, the *Solanaceae*, the *Asteraceae*, the *Malvaceae*, the *Fabaceae*, the *Linaceae*, the *Canabinaceae*, the *Dauaceae* and the *Cucurbitaceae* family, and which encode polypeptides homologous to MMR proteins of yeasts or humans may be obtained.

Examples of plants whose genes encoding polypeptides homologous to MMR proteins of yeasts or humans may be obtained given the teaching herein include maize, wheat, oats, barley, rice, tomato, potato, tobacco, capsicum, sunflower, lettuce, artichoke, safflower, cotton, okra, beans of many kinds including soybean, peas, melon, squash, cucumber, oilseed rape, broccoli, cauliflower, cabbage, flax, hemp, hops and carrot.

Within the meaning of the present invention, a first polypeptide is defined as homologous to a second polypeptide if the amino acid sequence of the first polypeptide exhibits a similarity of at least 50% on the polypeptide level to the amino acid sequence of the second polypeptide.

A procedure which may be followed to obtain genes *AtMSH3* and *AtMSH6* is described in Example 1. Essentially the same technique may be applied to obtain other mismatch repair genes of *Arabidopsis thaliana*, and essentially the same technique as exemplified herein may be applied to cDNA obtained by reverse transcription of RNA from other plants. Alternatively, given the sequence information disclosed herein, other degenerate oligonucleotide primers, especially oligonucleotides of the invention which are capable of hybridising at 45°C under standard PCR conditions (such as the conditions described in Example 1 using primers UPMU and DOMU) to *AtMSH3* and/or *AtMSH6* may be designed and obtained for use in isolating sequences of plant mismatch repair genes which are homologous to *AtMSH3* or *AtMSH6*, from other plants. Similarly, oligonucleotides of the invention which are capable of hybridising at 45°C under standard PCR conditions to plant mismatch repair genes of plants other than *Arabidopsis thaliana* also fall within the scope of the present invention and may be utilised to obtain mismatch repair genes of still other plants. Typically, such oligonucleotides are capable of hybridising at 45°C under standard PCR conditions to a DNA molecule which encodes a polypeptide which is homologous to a mismatch repair polypeptide of a yeast or a human. The temperature at which oligonucleotides of the invention hybridise to *AtMSH3* and/or *AtMSH6*, or to plant mismatch repair genes of plants other than *Arabidopsis thaliana*, or to DNA molecules which encode polypeptides which are homologous to a mismatch repair polypeptide of a yeast or a human may be higher than 45°C, for example at least 50°C, or at least 55°C, or at least 60°C or as high as 65°C.

The successful gene isolation disclosed herein demonstrates for the first time the existence of MMR in higher plants and indicates the presence of other plant MMR genes. For example, genes encoding the plant homologs of MSH1, MSH2, MSH4, MSH5, PMS1, PMS2 and MLH1 may be identified given the teaching herein. Such genes, as well as those specifically described herein, separately or in combination, are useful in manipulating the plant MMR for plant breeding purposes. Thus, for example, the plant MMR may be altered by including in a plant cell a polynucleotide sequence as defined herein above with reference to the third embodiment of the invention, and causing the polynucleotide sequence to express either a polynucleotide which disables a plant MMR gene, or a polypeptide which disrupts the plant's MMR system.

The DNA molecule of the third embodiment of the invention includes a polynucleotide sequence (herein referred to as a MMR altering gene) which may for example encode sense, antisense or ribozyme molecules characterised by sufficient base sequence similarity or complementarity to the gene to be altered to permit the antisense or ribozyme molecule to hybridise with the plant MMR gene *in vivo* or to permit the sense molecule to participate in co-suppression. Alternatively, the MMR altering gene may encode a protein or proteins which interfere with the activity of a plant MMR protein and thus disrupt the plant's MMR system. For example, such encoded proteins may be antibodies or other proteins capable of interfering with MMR protein function, such as by complexing with a protein functionally involved in plant MMR thereby disrupting the MMR of the plant. An example of such a protein is the MSH3 protein of *Arabidopsis thaliana* described herein or a protein of another plant which is homologous to the MSH3 protein of *A. thaliana*. For instance, overexpression of MSH3 in a plant cell causes MSH2 present in the cell to be substantially completely complexed, disrupting the mismatch repair mechanism or mechanisms in the cell which are functionally dependent on the presence of a complex of MSH2 with MSH6. Similarly, mismatch repair mechanisms which depend on the presence of a complex of MSH2 and MSH3 may be disrupted by the overexpression of MSH6.

A chimeric gene of the fourth embodiment, incorporating a MMR altering gene, may be prepared by methods which are known in the art. Similarly, the MMR altering gene may be introduced into a plant cell, regenerating tissue or whole plant by techniques known in the art as being suitable for plant transformation, or by crossing. Known transformation techniques include *Agrobacterium tumefaciens* or *A. rhizogenes* mediated gene transfer, ballistic and chemical methods, and electroporation of protoplasts.

The MMR altering gene or genes are typically expressed from suitable promoters. Suitable promoters may direct constitutive expression, such as the 35S or the *NOS* promoter. Usually, however, the promoter will direct either inducible or tissue specific (e.g. callus; embryonic tissue; etc.), cell type specific (e.g. protoplasts; meiocytes; etc.) or developmental (e.g. embryo) expression of the altering gene or genes, in order for the

MMR system to function in tissue types or cell types, or at developmental stages of the plant, in which it is not desirable for the MMR system to be altered. Using such promoters, therefore, the activity of a MMR altering gene may be limited to a specific stage during plant development or it may be altered by controlling conditions external to the plant, and the deleterious effects of a permanently disabled or altered DNA mismatch repair system in a plant may be avoided. Examples of suitable promoters which are not constitutive are known in the art and include inducible promoters such as *PR1a* (reviewed by Gatz, 1997, Annual Rev. Plant Phys. Plant Mol. Biol. 48: 89), tissue specific promoters such as *AoPRI* (Sabahattin et al., 1993, Biotechnology 11: 218), and cell-type specific promoters such as *DMC1*.

A chimeric gene in accordance with the invention may further be physically linked to one or more selection markers such as genes which confer phenotypic traits such as herbicide resistance, antibiotic resistance or disease resistance, or which confer some other recognisable trait such as male sterility, male fertility, grain size, colour, growth rate, flowering time, ripening time, etc.

The process of the tenth embodiment of the invention provides, for example, a process for generating intraspecies genetic variation by altering the mismatch repair system in a plant cell, in regenerating plant tissue or in a whole plant. The plant cell, regenerating tissue or whole plant includes and expresses one or more MMR altering genes which are capable of altering mismatch repair in the plant cell, regenerating tissue or whole plant. Alteration of MMR may be achieved, for example, by inactivating the genes encoding plant MSH3 and/or plant MSH6. It is preferred to inactivate the plant MSH3 and MSH6 encoding genes at the same time and in the same plant cell, regenerating tissue or whole plant. Typically in this preferred form of the invention inactivation of either plant MSH3 or MSH6 alone is insufficient to substantially alter the plant's mismatch repair system and only when both MSH3 and MSH6 are inactivated simultaneously is the plant's mismatch repair system sufficiently altered to prevent the MMR system from recognising base pair mismatches, base insertions or deletions as a result of DNA replication errors, DNA damage, or oligonucleotide induced site-specific mutagenesis. However, in some applications of the invention, inactivation of only one gene may also be used to cause genomic instability or increase the efficiency of site-specific mutagenesis.

If desired, the MMR altering gene or genes may later be rendered non-functional or ineffective, or may be removed from the genome of the plant cell, regenerating tissue or whole plant in order to restore mismatch repair in the plant cell, regenerating tissue or whole plant. The MMR altering gene or genes may be inactivated by means of known gene inactivation tools, such as ribozymes, or may be removed from the genome using gene elimination systems known in the art, such as *CRE/LOX*. It is preferred to render two genes, whose gene products combine to incapacitate MMR, ineffective by separating

the altering genes through segregation. Therefore, in a preferred embodiment of the invention a first plant cell or plant is generated in which only plant *MSH3* is incapacitated, and a second plant cell or plant is generated in which only plant *MSH6* is incapacitated. The combination of both genomes, for example by crossing, then produces significant 5 MMR deficiency in those cells or plants which have inherited both altering genes. If the altering genes are expressed from unlinked loci, gene segregation restores MMR activity in the progeny of the cells or plants.

In a process of the ninth embodiment of this invention, homeologous recombination is enhanced between different genomes, chromosomes or genes in plant cells or plants by 10 altering MMR in said plant cells or plants. Such genomes, chromosomes or genes are characterised in that they originate from different plant families, genera, species, subspecies, plant varieties or lines. Hybrid plant cells or hybrid plants may be produced by crossing, by cell fusion or by other techniques known in the art. These plant cells or plants are further characterised by expressing one or more genes that are capable of 15 altering mismatch repair in the plant cell or plants.

In the process of the ninth embodiment, the homeologous recombination is typically for the purpose of introducing a desired characteristic in the hybrid plant. In this typical application of the process of the ninth embodiment, and in the process of the tenth embodiment the desired characteristic may be any characteristic which is of value to the 20 plant breeder. Examples of such characteristics are well known in the art and include altered composition or quality of leaf or seed derived storage products (e.g. oil, starch, protein), altered composition or quality of cell walls (e.g. decrease in lignin content), altered growth rate, prolonged flowering, increased plant yield or grain yield, altered plant morphology, resistance to pathogens, tolerance to or improved performance under 25 environmental stresses of various kinds, etc.

In a preferred form of the tenth embodiment, an MMR altering gene is co-introduced along with the homeologous genome, chromosome or gene of another plant cell or plant into an MMR proficient plant cell or MMR proficient plant to produce a hybrid plant cell or hybrid plant in which homeologous recombination can occur. 30 Suitably, the MMR proficient plant cell or MMR proficient plant may also include an MMR altering gene. For example a gene capable of inactivating plant *MSH3* may be co-introduced along with the homeologous genome, chromosome or gene of another plant cell or plant into an MMR proficient plant cell or MMR proficient plant in which *MSH6* is inactivated. A resultant hybrid plant in which homeologous recombination occurs will 35 include both the *MSH3* and *MSH6* altering genes and its MMR system will therefore be inactivated.

In this form of the invention, if hybrid plants are to be produced by crossing, the MMR altering gene preferably originates from the male parent, thus ensuring that the

MMR altering gene is always introduced and is not present in the recipient cell. That is, the MMR of the recipient cell, prior to introduction of the MMR altering gene, is typically proficient. Alternatively, if an MMR altering gene is present in a recipient cell it may be ineffective or inefficient on its own, or it may be linked to an inducible or tissue specific or cell type specific promoter which only renders the MMR altering gene active under limited conditions.

Thus, in a preferred form of the process of the ninth embodiment, the MMR system of the hybrid plant is initially unaltered. In this form of the process, the step of altering the mismatch repair system may comprise introducing into the hybrid plant, or cells thereof, a MMR altering gene, such as by *Agrobacterium tumefaciens* or *A. rhizogenes* mediated gene transfer, ballistic and chemical methods, and electroporation of protoplasts.

The MMR altering gene or genes are typically expressed from suitable promoters, as described above. Preferably, the promoter is transcriptionally active in mitotically and meiotically active tissue and/or cells to ensure MMR alteration after chromosome pairing at mitosis and meiosis, respectively. The preferred timing for MMR alteration is at meiosis, because recombinant genomes, chromosomes or genes are directly transmitted to the progeny. A suitable meiocyte specific promoter is for example the *DMC1* promoter from *Arabidopsis thaliana* ssp. *Ler*. (Klimyuk and Jones, 1997, Plant J. 11, 1-14). However, mitotic homeologous recombination is also a desirable outcome as somatic recombination events can be transmitted to offspring due to the totipotency of plant cells and the lack of predetermined germ cells in plants.

If desired, the MMR altering gene or genes may later be rendered non-functional or ineffective, or may be removed from the hybrid plant or hybrid plant cells, in order to restore mismatch repair in the hybrid plant or hybrid plant cells. The MMR altering gene or genes may be inactivated by means of known gene inactivation tools as described herein above.

## EXAMPLES

### Example 1. Cloning of the *AtMSH3* and *AtMSH6* coding sequences

#### Isolation of partial *AtMSH3* and *AtMSH6* consensus sequences

Degenerate oligonucleotides UPMU (SEQ ID NO:1) and DOMU (SEQ ID NO:2)

UPMU	CTGGATCCACIGGGICCIAA(C/T)ATG
DOMU	CTGGATCC(A/G)TA(A/G)TGIGTI(A/G)C(A/G)AA

were used to isolate *AtMSH3* and *AtMSH6* sequences by PCR amplification.

Primers UPMU and DOMU correspond to conserved amino acid sequences of the proteins MutS (*E. coli* and *S. typhimurium*), HexA (*S. pneumoniae*), Rep1 (mouse) and Duc1 (human). The conserved regions to which they are targeted are TGPNM for UPMU (amino acid positions 852-856 for AtMSH6 and 816-820 for AtMSH3) FATHY or FVTHY

for DOMU (amino acid positions 964-968 for AtMSH6 and 928-932 for AtMSH3, respectively.) These primers have been used to isolate MSH2 and MSH1 from yeast (Reenan and Kolodner, Genetics 132: 963-973 (1992)) and MSH2 from *Xenopus* and mouse (Varlet et al., Nuc. Acids Res. 22:5723-5728 (1994)).

5      Template single strand cDNA was produced by reverse transcription of 2 µg total RNA from a cell suspension culture of *Arabidopsis thaliana* ecotype Columbia (Axelos et al. 1989, Mol. Gen. Genetics 219: 106-112). The PCR reaction was performed under the following conditions in a final volume of 100µl: 0.2mM dNTP, 1µM each primer, 1XPCR buffer, 1u *Taq* DNA polymerase (Appligene) in the presence of template cDNA. PCR parameters were 5 minutes at 94°C, followed by 30 cycles of 40 seconds at 95°C, 90 seconds at 45°C, 1 minute at 72°C. The amplification products were cloned into pGEM-T vector (Promega) and sequenced. Two different clones were isolated, S5 (350bp) was homologous to *MSH3*, S8 (327bp) was homologous to *MSH6*. Complete cDNA sequences were then isolated according to the Marathon cDNA amplification kit procedure (Clontech).

10     In summary, this procedure involves producing double stranded cDNA by reverse transcription of 2µg polyA+ RNA from the cell suspension culture of *Arabidopsis*. Adaptors are ligated on each side of the cDNA. The ligated cDNA is used as a template for 5' and 3' RACE PCR reactions in the presence of primers that are specific for the adaptor on one side (AP1 and AP2), and specific for the targeted gene on the other side. A 5' and a 3' fragment that overlap are thus produced for each gene. The complete gene coding sequence can be reconstituted taking advantage of a unique restriction site, if available, in the overlapping region. Specific details of this procedure as it was used to isolate *AtMSH3* and *AtMSH6* coding regions, are as follows.

15     20     25

#### Isolation of *AtMSH3* complete coding sequence

From the sequence of clone S5, primer 636 (SEQ ID NO:3) was designed:

636     TGCTAGTGCCTCTTGCAAGCTCAT.

Primer AP1 (SEQ ID NO:4) is complementary to a portion of an adaptor sequence which had been ligated to the 5' and 3' ends of *Arabidopsis* cDNA:

AP1     CCATCCTAACATCGACTCACTATAGGGC.

30     PCR performed on the ligated cDNA with primers 636 and AP1 for the 5' RACE PCR was followed by a second round of amplification with the nested primers AP2 (SEQ ID NO:5) and S525 (SEQ ID NO:6)

AP2     ACTCACTATAAGGGCTCGAGCGGC

S525     AGGTTCTGATTATGTGTGACGCTTACTTA

35     (the latter was also designed to correspond to a part of the sequence of clone S5) and produced a 2720bp DNA fragment. Figure 1 provides a diagrammatic representation of the primer sequences used to isolate *AtMSH3*. Another primer (S51, SEQ ID NO:7)

S51     GGATCGGGTACTGGGTTTGAGTGTGAGG

was designed closer to the 5' border and permitted the determination of 99bp upstream to the ATG initiation codon. For the 3' RACE PCR, a first PCR reaction was performed with primers AP1 and 635 (SEQ ID NO:8).

635            GCACGTGCTTGTGGTGTTCAC

5 followed by a second round of amplification, using the nested primers AP2 and S523 (SEQ ID NO:9)

S523            TCAGACAGTATCCAGCATGGCAGAAGTA

which produced a DNA fragment of 890bp. Both DNA fragments were subcloned into pGEM-T and sequenced. Since PCR amplification using the Expand Long Template PCR 10 System (Boehringer-Mannheim) produced errors in the sequence, new oligonucleotides were designed to isolate those sequences again by PCR, but with the high fidelity DNA polymerase *Pfu*. PCR with primers 1S5 (SEQ ID NO:10) and S53 (SEQ ID NO:11)

1S5            ATCCCGGGATGGGCAAGCAAAAGCAGCAGACGA

S53            GACAAAGAGCGAAATGAGGCCCTTGG

15 amplified the 1244bp fragment clone 52 (SEQ ID NO:12, cloned into pUC18/Sma1). PCR with primers S52 (SEQ ID NO:13) and 2S5 (SEQ ID NO:14)

2S5            ATCCCGGGTCAAAATGAACAAGTTGGTTTAGTC

S52            GCCACATCTGACTGTTCAAGCCCTCGC

amplified the 2104bp clone 13 (SEQ ID NO:15, cloned into pUC18/Sma1). The complete 20 coding sequence of the *AtMSH3* gene was reconstructed in pUC18 by ligating the 5' half of *AtMSH3* (clone 52) to the 3' half of *AtMSH3* (clone 13) after digesting with *Bam*H1 which has a unique cleavage site in the overlapping region of both clones. This manipulation yielded plasmid pPF26. The *Sma*1 fragment from pPF26 contains the complete *AtMSH3* coding sequence. The remaining primers referred to in Figure 1 are as 25 follows:

S51            GGATCGGGTACTGGGTTTGAGTGTGAGG (SEQ ID NO:16)

S525            AGTTCTGATTATGTGTGACGCTTTACTTA (SEQ ID NO:17)

Figures 2 and 3 provide plasmid maps of clones 52 and 13 respectively, showing 30 restriction enzyme cleavage sites. The complete *AtMSH3* coding sequence (SEQ ID NO:18) is 3246bp long and is shown in Figure 4 together with the deduced sequence (SEQ ID NO:19) of the encoded polypeptide. *AtMSH3* is clearly homologous to the yeast and mouse *MSH3* genes. A sequence alignment of polypeptides encoded by *AtMSH3* and that encoded by *Saccharomyces cerevisiae MSH3* is set out in Figure 5.

#### Isolation of the *AtMSH6* complete coding sequence and genomic sequences

35 The same procedure allowed isolation of the *AtMSH6* cDNA. Figure 6 provides a diagrammatic representation of the primer sequences used to isolate *AtMSH6*. For the 5' RACE PCR, primers 638 (SEQ ID NO:20) and AP1 (SEQ ID NO:4)

638            TCTCTACCAGGTGACGAAAAACCG

allowed the amplification of a 2889 DNA fragment. Primer S81 (SEQ ID NO:21)

S81 CGTCGCCCTTAGCATCCCCCTTCCTTCAC

helped define the 142bp upstream to the ATG initiation codon. On the 3' side, RACE PCR was initially performed with primers S823 (SEQ ID NO:22) and API (SEQ ID NO:4),

S823 GCTTGGCGATCTAATAGAACATGACAGG

5 and then with the nested primers 637 (SEQ ID NO:23) and AP2 (SEQ ID NO:5).

637 GACAGCGTCAGTTCTTCAGAACATGC

to produce a 774bp DNA fragment. As for *AtMSH3*, those fragments were cloned and sequenced. Re-isolation of the DNA sequence using the high fidelity *Pfu* polymerase and newly designed primers 1S8 (SEQ ID NO:24) and S83 (SEQ ID NO:25) (for the 5' side) led 10 to a 2182 bp DNA fragment identified as clone 43 (SEQ ID NO:26, cloned in pUC18/Sma1), and a 1379bp clone identified as clone 62 (SEQ ID NO:27, also cloned in pUC18/Sma1).

1S8 ATCCCGGGATGCAGCGCCAGAGATCGATTTGT

2S8 ATCCCGGGTTATTGGAAACACAGTAAGAGGATT (SEQ ID

15 NO:28)

S82 GCGTCGATCATCAGCCTCTGTGTTGC (SEQ ID NO:29)

S83 CGCTATCTATGGCTGCTTCGAATTGAG

Figures 7 and 8 provide plasmid maps of clones 43 and 62 respectively, showing restriction enzyme cleavage sites. Clones 43 and 62 were digested by the *Xmn*1 restriction enzyme for 20 which a unique site is present in their overlapping region and then ligated. The complete *AtMSH6* coding sequence (SEQ ID NO:30) is 3330bp long and is shown in Figure 9 together with the deduced sequence (SEQ ID NO:31) of the encoded polypeptide. *AtMSH6* is clearly homologous to the yeast and mouse *MSH6* genes. A sequence alignment of 25 polypeptides encoded by *AtMSH6* and that encoded by *Saccharomyces cerevisiae MSH6* is set out in Figure 10.

An *AtMSH6* genomic sequence was also isolated from a genomic DNA library constituted after partial *Sau3AI* digestion of DNA from the *Arabidopsis* cell suspension. 8062bp were sequenced that covered the *AtMSH6* gene and show colinearity with the cDNA. 16 introns are found scattered along the gene. The complete genomic sequence 30 (SEQ ID NO:98) is shown in Figure 11.

#### **Example 2. A measure of somatic variation in MMR deficient plants**

##### Constructs

Constructs with antisense *AtMSH3* or antisense *AtMSH6* or both *AtMSH3/AtMSH6* under the control of a single 35S promoter have been inserted into the binary vector 35 pPZP121 (Hajdukiewicz et al., Plant Mol. Biol. 23, 793-799) between the right and left borders of the T-DNA. The pPZP121 plasmid confers chloramphenicol resistance to *Escherichia coli* or *Agrobacterium tumefaciens* bacteria. The *aacC1* gene is carried by the T-DNA and allows selection of transformed plant cells on gentamycin (Hajdukiewicz et al., Plant Mol. Biol. 25, 989-994). For the purpose of expressing antisense constructs, a 35S

promoter/terminator cassette with a polylinker was introduced into pPZP121. The 3' ends of the considered genes have been chosen since this region seems more efficient for antisense inhibition. For *AtMSH3* this corresponds to clone 13 (2104bp), for *AtMSH6* this corresponds to clone 62 (1379bp). Clone 13 comprises 2104bp of the 3' region that were cut off the pUC18 vector by SalI/SstI restriction, blunted with T4 DNA polymerase and ligated into the T4 DNA polymerase blunted *Bam*HI site of pPZP121/35S, creating clone pPF13. The same procedure was followed for the 3' region of *AtMSH6* clone 62 (1379bp) thus creating plasmid pPF14. For the double constructs, the 3' region (from clone 62) of *AtMSH6* was introduced ahead of the *AtMSH3* region into pPF13 creating pCW186 and reciprocally, the 3' region of *AtMSH3* (from clone 13) was introduced ahead of *AtMSH6* into pPF14, creating pCW187.

These constructs were introduced into the Arabidopsis cells (as described below) of wildtype Columbia and of the Columbia tester line.

An alternative strategy to antisense inhibition of *AtMSH6* comes from experiments of Marra et al. (1998. Proc. Natl. Acad. Sci USA 95. 8568-8573) who show that overexpression of functional *MSH3* results in depletion of MSH6 protein in human cells. This depletion may generate a mismatch repair mutant phenotype.

For the purpose of overexpressing functional *AtMSH3* protein in plant cells, the complete *MSH3* coding region was excised from pPF26 (example 1) by digestion with *Sma*I, and was inserted into the *Sma*I site of pCW164. The resulting construct was named pPF66. It contains a complete *AtMSH3* gene under the control of the 35S promoter inside the left (LB) and right (RB) border of the T-DNA. This T-DNA also contains the *hpt2* gene for gentamycin selection. Plasmid pPF66 was introduced into Arabidopsis cells as described below. One cell clone was selected which clearly overexpressed the *AtMSH3* gene as shown by Northern analysis. Figures 12-16 provide plasmid maps of plasmids pPF13, pPF14, pCW186, pCW187 and pPF66, respectively.

#### Construction of tester construct

For the purpose of Forward Mutagenesis Assays, a tester construct was built containing the coding regions for *nptII*, *codA*, *uidA*. All three genes are driven by the 35S promoter and are terminated by the 35S terminator. This construct was obtained by introducing an EcoR1 fragment encoding the *codA* cassette (2.5kb) and a *Hind*III fragment encoding the *uidA* (*GUS*) cassette (2.4kb) into the pPZP111 vector (Hajdukiewicz et al., 1994, Plant Mol Biol 23: 793-799) which already contained the *nptII* expression cassette. This new plasmid was named pPF57. *NptII* is used to select for transformed plant cells. *GUS* is used to analyse the degree of gene silencing in the construct (i.e. to identify cell lines in which the transgenes are expressed), and *codA* is used as a marker for forward mutagenesis (described below).

The plasmid map of pPF57 is provided in Figure 17.

#### Plant cell transformation

The constructs are introduced into *Agrobacterium* by electroporation. Plant cells are then transformed by co-cultivation. A suspension culture of *Arabidopsis thaliana* cells that has been established by Axelos et al. (1992, Plant Physiol. Biochem. 30, 1-6) may be used. One day old freshly subcultured cells are diluted five times into AT medium (Gamborg B5 medium, 30g/l sucrose, 200 $\mu$ g/l NAA). 10 $\mu$ l of saturated *Agrobacterium* containing the transforming T-DNA constructs are added to 10ml diluted cells in a 100ml erlenmeyer. The co-cultivation is agitated slowly (80rpm) for 2 days. The cells are then washed 3 times into AT medium and finally resuspended in the same initial volume (10ml). The culture is agitated for 3 days to allow expression before plating on selection plates (AT/BactoAgar 8g/l+gentamycin 50 $\mu$ g/ml). Transformed individual calli are isolated 3 weeks later.

#### Tester Strain

The tester construct on plasmid pPF57 was introduced into *Arabidopsis* cells of wildtype Columbia using the transformation protocol described above. Among 10 candidate transformants, one cell clone was shown (by Southern analysis) to have a unique T-DNA insertion. All three genes were shown to be functional in this cell line as indicated by resistance to kanamycin, blue staining in the presence of X-Glu (*GUS*), and sensitivity to 5-fluoro-cytosine (*codA*).

MMR altering genes (described above) were then introduced individually into the tester line and transformed cells are used for analysis of both Microsatellite Instability and Forward Mutagenesis.

#### Microsatellite analysis

Microsatellites have been described in *Arabidopsis* (Bell and Ecker, 1994, Genomics 19, 137-144). The present Example is based on a study of instability of microsatellites that are polymorphic amongst different ecotypes. DNA is extracted from the transformed calli. Specific primers have been defined that are used to amplify the microsatellite sequence. One of the two primers is previously P<sup>32</sup> labelled by T4 kinase. In case of a polymorphic variation, new PCR products appear that do not follow the expected pattern of migration on a polyacrylamide gel. This is a commonly observed feature for MMR deficient cells in yeast or mammalian cells.

In particular, the present Example describes a study on microsatellites ca72 (CA<sub>18</sub>), nga172 (GA<sub>29</sub>), and ATHGENEA(A<sub>39</sub>), chosen because they belong to the types predominantly affected in human mismatch repair deficient tumors. The size of these microsatellites is not conserved from one *Arabidopsis* ecotype to the other.

*Arabidopsis* cells which are transformed with an MMR altering gene (above) and control cells not expressing the MMR altering gene are allowed to form calli. DNA is

rapidly extracted from the calli and is analysed for microsatellite instability as described in detail by Bell and Ecker 1994. Genomics 19. 137-144. In summary, the relevant microsatellite is amplified by PCR using  $\alpha$ -<sup>32</sup>P labelled primers. The PCR products are separated on a DNA sequencing gel for size determination. Size differences between 5 microsatellites from transformed and control cells not expressing the MMR altering gene in question indicate microsatellite instability as a result of MMR alteration.

The sequences of primers used for PCR amplification of microsatellites ca72 and nga172 are included in Table 1. PCR amplification of microsatellite ATHGENEA made use of a forward primer containing the sequence

10 ACCATGCATAGCTTAAACTTCTTG (SEQ ID NO:32)

and of a reverse primer containing the sequence

ACATAACCACAAATAGGGGTGC (SEQ ID NO:33).

The amplification for microsatellite ca72 revealed in Columbia control cells (with respect to the MMR altering gene) a 248 bp long PCR fragment instead of the published 15 length of 124 bp. DNA sequencing verified this fragment as a CA<sub>18</sub> microsatellite.

#### Forward mutagenesis assay

Tester cells transformed with antisense *AtMSH3* or antisense *AtMSH6* or both *AtMSH3/AtMSH6* are analysed for the stability of the *codA* gene. The functional *codA* gene confers to sensitivity to 5-fluoro-cytosine (5FC), whereas a gene inactivating mutation in 20 *codA* will confer resistance to 5FC. The frequency of resistant cells is therefore a good indicator of somatic variation as a direct result of MMR alteration. Variants resistant to 5FC are first analysed for GUS activity. If GUS is inactive, 5FC resistance is assumed to be due to gene silencing (all three genes are under the 35S promoter). If GUS is active, 5FC resistance is assumed to be due to forward mutations that have inactivated *codA*. PCR is 25 then performed on the putative *codA* mutant genes which is then sequenced to confirm the presence of forward mutations in *codA*.

Besides *codA*, other marker genes may also be used for the Forward Mutagenesis Assay such as the *ALS* gene (conferring sensitivity to valine or to sulfonylurea; Hervieu and Vaucheret, 1996, Mol. Gen. Genet. 251 220-224; Mazur et al. 1987, Plant Physiol. 85 1110-30 1117).

#### **Example 3. Homeologous meiotic recombination in *Arabidopsis thaliana***

##### A. Construction of a meiocyte specific gene expression cassette comprising the *DMC1* promoter and the *NOS* terminator

(i) The *DMC1* promoter may be used as published by Klimyuk and Jones, 1997, 35 Plant J. 11.1-14). To obtain a more convenient alternative for gene cloning, a 3.3 Kb

long subfragment of the *DMC1* promoter was obtained by PCR from genomic DNA of *Arabidopsis thaliana* (ssp. Landsberg erecta "Ler").

The PCR was done in three rounds:

Round One: A 3.7 Kb long product was obtained using the forward primer  
5 DMCIN-A comprising the sequence

GAAGCGATATTGTCGTG (SEQ ID NO:34)

and the reverse primer DMCIN-B comprising the sequence

AGATTGCGAGAACATTCC (SEQ ID NO:35).

The weak amplification product was then used as template for round two and three.

10 Round Two: A 3.1 Kb long product comprising the promoter and the 5' untranslated leader was obtained using forward primer DMCIN-1, which contained the sequence

acgcgtcgacTCAGCTATGAGATTACTCGTG (SEQ ID NO:36)

and introduced a *Sal*I cloning site at the 5' end of the promoter fragment, and reverse  
15 primer DMCIN-2 which contained the sequence

gctctagaTTTCTCGCTCTAAGACTCTCT (SEQ ID NO:37)

and introduced a *Xba*I site at the 3' end of the PCR fragment.

Round Three: A 0.2 Kb long product comprising the first exon/intron of the *DMC1* promoter was obtained using forward primer DMCIN-3, which contained the sequence

20 gctctagaGCTTCTCTTAAGTAAGTGATTGAT (SEQ ID NO:38)

and introduced a *Xba*I site at the 5' end of the PCR fragment, and reverse primer DMCIN-4, containing the sequence

tccccccgggctcgagagatctccatggTTTCTTCAGCTCTATGAATCC (SEQ ID NO:39)

and introduced at the 3' end of the PCR product restriction sites for *Nco*I, *Bgl*II, *Xho*I and  
25 *Sma*I.

The products obtained in round Two and Three were digested with *Xba*I and subsequently ligated to reconstitute a 3.3 Kb long *DMC1* promoter from which the first two in-frame ATG start codons were replaced with a unique restriction site for *Xba*I. This promoter can be cloned between the restriction sites for *Sal*I and *Sma*I of p3264, 30 which contains the *Sac*I-*Eco*RI NOS terminator in pBIN19, to yield the entire expression cassette in pBIN19. This cassette contains the following cloning sites: *Nco*I, *Bgl*II, *Xho*I, *Sma*I and (already present on p3264) *Kpn*I and *Sac*I.

(ii) Another strategy yielded the following convenient *DMC1* promoter. A 1.8 kb DNA fragment comprising the 3' terminal part of the meiocyte specific *DMC1* promoter 35 was isolated by PCR from purified genomic DNA of *Arabidopsis thaliana* (ssp. Landsberg erecta "Ler"). The forward PCR primer (DMC1a) contained the sequence

acgcgtcgacGAATTCTCGCAAGTGGGG (SEQ ID NO:40)

and introduced a *Sal*I cloning site at the 5' end of the promoter fragment. The reverse PCR primer (DMC1b) contained the sequence

tcctatggagatctccgggtacCGATTTGCTTCGAGGG (SEQ ID NO:41)

introducing a polylinker region at the 3' end of the promoter fragment. The PCR reaction was carried out with VENT DNA Polymerase (NEB) over 25 cycles using the following cycling protocol: 1 minute at 94°C, 1 minute at 54°C, 2 minutes at 72°C.

5 The PCR reaction yielded a blunt ended DNA fragment which was digested with restriction enzyme *Sall* and was cloned into the cleavage sites of restriction enzymes *Sall* and *SmaI* in plasmid p2030, a pUC118 derivative containing the *SacI-EcoRI* NOS terminator fragment of pBIN121. The cloning yielded plasmid p2031, containing the *DMC1* promoter-polylinker-NOS terminator expression cassette depicted in Figure 18.

10 B. Construction of an *MSH3* antisense gene under the control of the *DMC1* promoter

A 2.1 kb DNA fragment encoding the carboxyterminal part of AtMSH3 was removed from the polylinker of clone 13 described in Example 1 after (i) digestion with *KpnI*, (ii) blunting of the DNA ends generated by *KpnI* and (iii) digestion with *BamHI*. The isolated fragment was then cloned in antisense orientation downstream of the *DMC1* promoter in plasmid p2031, which had been digested with restriction enzymes *SmaI* and *BgII*. This cloning yielded plasmid p2033 (Figure 18).

After digestion of p2033 with *EcoRI*, a 4.1 kb DNA fragment was recovered comprising the *DMC1* promoter, the partial *MSH3* cDNA in antisense orientation with respect to the promoter and the *NOS* terminator. This fragment was cloned into the *EcoRI* 20 restriction site of plant transformation vector pNOS-Hyg-SCV to yield plasmid p3242 (Figure 18).

C. Construction of a combined *MSH6/MSH3* antisense gene under the control of a single *DMC1* promoter

A 3.1 kb fragment, encoding in antisense orientation the partial *AtMSH6* and *AtMSH3* sequences and the 35S terminator, was isolated from pCW186 by digestion with *KpnI*. The fragment was treated with *Klenow* enzyme to blunt both ends. It was then cloned into the *SmaI* site of plasmid p3243 (a pNOS-Hyg-SCV derivative, illustrated in Figure 19), which had been opened to delete the region between the *SmaI* sites. Clones containing the fragment in the antisense orientation with respect to the *DMC1* promoter (described in 30 A(ii) above) were identified by diagnostic digestion with *BamHI*. The selected construct was named p3261.

Another practical way of cloning the double antisense gene is as follows. A 1 kb DNA fragment encoding the carboxyterminal part of AtMSH6 is isolated from clone 62 described in Example 1 after digestion of clone 62 plasmid DNA with *BamHI*, which 35 cleaves in the 5' polylinker region flanking the partial cDNA, and with *EcoRI*, which cleaves within the cDNA. The isolated fragment is treated with *Klenow* enzyme to blunt both its ends and is cloned into the recipient plasmid p2033 or p3242. For the purpose of

cloning, the recipient plasmid may be cleaved with either *Ava*I or *Nco*I and can be blunted with *Klenow* enzyme to produce blunt acceptor ends for fragment cloning. This cloning yields two opposite orientations of cloned fragment DNA with respect to the *DMC1* promoter. These can be identified by diagnostic digestion with restriction enzymes *Scal* 5 or *Xmn*I in conjunction with *Sac*I. The selected construct contains the *DMC1* promoter, the combined partial cDNAs for *AtMSH3* and *AtMSH6* (both cloned in antisense orientation with respect to the *DMC1* promoter) and the *NOS* terminator. If the recipient plasmid is p2033, the combined antisense gene under control the single *DMC1* promoter is recovered from the vector after *Eco*RI digestion and is cloned into the *Eco*RI restriction 10 site of pNOS-Hyg-SCV.

D. Construction of a full-length *MSH3* sense gene under control of the *DMC1* promoter for overexpression of functional *MSH3* protein

Overexpression of *MSH3* protein was shown in human cells (Marra et al., 1998, Proc. Natl. Acad. Sci. USA 95, 8568-8573) to complex all available *MSH2* protein. This 15 leaves *MSH6* protein without its partner, leading to the degradation of *MSH6* protein and eventually to a mismatch repair phenotype.

This phenomenon is exploited to increase homeologous meiotic recombination in Arabidopsis as an alternative to antisense inhibition of *AtMSH6*. For this purpose the full-length cDNA encoding *AtMSH3* is isolated from plasmid pPF66 by digestion with *Sma*I 20 and is cloned into the *Sma*I site of the *DMC1* expression cassettes described in A(i).

E. Selection of Recombination markers on homeologous chromosomes of *Arabidopsis thaliana* subspecies *Landsberg erecta* (Ler), *Columbia* (Col) and C24, respectively

E(i). Visual recombination markers in *Arabidopsis th. subspecies C24*:

*Agrobacterium* mediated transformation with a T-DNA containing a 35S-*GUS* gene, 25 inactivated by insertion of a 35S-*Ac* transposable element (Finnegan et al., 1993, Plant Mol. Biol. 22, 625-633), had yielded a C24 line in which the T-DNA construct was integrated into chromosome 2. Genetic and molecular analysis of this line shows that the *Ac* transposon had excised from its T-DNA locus thereby restoring *GUS* activity, but had re-inserted into the chromosome at a distance of 16.4 cM, where it stayed fixed (due to 30 disablement of *Ac*) within the *chlorina* gene. Insertional inactivation of the *chlorina* gene caused a bleached phenotype in those plants that were homozygous for this mutation. Because of the two linked phenotypic markers, *chlorina3A:Ac* and *GUS*, this C24 line was used in crosses to wildtype Ler for analysis of meiotic homeologous recombination on chromosome 2 in conjunction with molecular recombination markers.

E(ii). Visual recombination markers in *Arabidopsis th. Ler*:

The Ler line NW1 (obtained from NASC, Nottingham, UK) contains one recessive visual marker per chromosome, i.e. *an-1* on Chr.1, *py-1* on Chr.2, *gll-1* on Chr.3, *cer2-1*

on Chr.4, and *msl-1* on Chr.5. This line is used in crosses to wildtype C24 which expresses an MMR altering gene for analysis of meiotic homeologous recombination on chromosomes 1-5 in conjunction with molecular recombination markers listed in Table 1.

Other *Ler* lines from NASC have several visual markers in close proximity to each other on the same chromosome. When these lines are used for hybrid production, analysis of homeologous meiotic recombination may make use entirely of visual recombination markers. Particularly suitable for crossing to C24 wildtype that is expressing a MMR altering gene are the following *Ler* lines:

- NW22: relative markers are *dis1* - (4 cM) - *ga4* - (11 cM) - *th1* on chromosome 1.  
10 NW10: relevant markers are *tz-201* - (5 cM) - *cer3* on chromosome 5.  
NW134, relevant markers are *ttg* - (4 cM) - *ga3* on chromosome 5.

NW24 (*abi3-1*) and NW64 (*gl1-1*). When present in the same plant on chromosome 3, *abi3-1* and *gl1-1* are 13 cM apart. Since this marker combination is not available from NASC, we have combined these markers by crossing line NW24 to line NW64. The F1 15 offspring were allowed to self-fertilise and to produce F2 seeds. F2 Plants which carry both markers as homozygous traits on the same chromosome can be identified firstly, by germinating F2 seeds on germination medium containing selective concentrations of abscisic acid, and subsequently, by identifying among the abscisic acid resistant plants those individuals which show the glabra phenotype.

20 E(iii) Molecular recombination markers in *Col*, *Ler* and *C24*:

The genome of *Arabidopsis thaliana* is interspersed with unique base sequences arranged as simple tandem repeats. Allelic repeats can vary in length between different *Arabidopsis* subspecies and when amplified by PCR yield diagnostic DNA products of different length named Simple Sequence Length Polymorphisms (SSLPs). Many SSLPs 25 have been genetically mapped and have been assigned to unique chromosome locations on the recombinant inbred map (Bell and Ecker, 1994, Genomics 19, 137-144; Lister and Deans lines, Weeds World 4i, May 1997).

In Table 1 are listed 28 mapped and established SSLPs between *Ler* and *Col*. A number of PCR primer pairs are described herein (SEQ ID NO:42 to SEQ ID NO:97) 30 which also yielded SSLPs between *C24* and *Ler* (19 SSLPs) or between *C24* and *Col* (25 SSLPs), respectively. Polymorphic SSLPs can be used as molecular markers in the analysis of homeologous recombination between genomes from these subspecies.

The PCR reactions (25 µL) were carried out over 35 cycles (15 seconds at 94°C, 30 seconds at 55°C and 30 seconds at 72°C), with 0.25 U Taq DNA polymerase and 0.6 µg 35 genomic DNA in reaction buffer containing 2 mM MgCl<sub>2</sub>. PCR products were separated by agarose gel electrophoresis (4% ultra high resolution agarose) and visualised by ethidiumbromide staining. The results from the PCR experiments are summarised in

Table 1, which also shows the sequence of PCR primers, primer annealing temperature ( $T_m$ ), PCR product length and chromosome location of SSLP (with respect to the RI map of May 1997, Weeds World 4i).

F. Production of hybrid plants

5      C24 plants heterozygous for *chlorina3A:Ac/GUS* are crossed as male to emasculated wildtype *Ler* to produce *Ler/C24(chlorina3A, GUS)* hybrid seeds.

Due to the heterozygosity of the C24 parent, only 50 % of hybrid plants have inherited the *chlorina3A:Ac/GUS* locus. The remaining 50% of hybrid plants are wildtype with respect to *chlorina3A:Ac/GUS*. Since the mutant locus is linked to a kanamycin 10 resistance gene (contained on the same T-DNA as *GUS*) mutant plants can be pre-selected by germinating hybrid seeds on germination medium containing 50 mg/L kanamycin.

*Ler* plants homozygous for the five chromosome markers are male sterile (*ms1-1*) and are crossed without emasculation to wildtype C24 to produce *Ler(an-1, py-1, gll-1, cer2-1, ms1-1)/C24* hybrid seeds.

15      Other *Ler* plants, which are male fertile, are crossed after emasculation of the female parent to produce *Ler/C24* hybrid seeds.

G. Introduction of *MSH3* and *MSH6/3* antisense genes into *Arabidopsis* and analysis of meiotic homeologous recombination

(i) Transformation of hybrid plants and analysis of homeologous meiotic recombination

20      The plant transformation vectors comprising the antisense genes described in (B) and (C) above are introduced into *Agrobacterium tumefaciens* strain AGL1 (Lazo et al., 1991, Bio/Technology 9, 963-967) by electroporation. Recombinant *Agrobacterium* clones are selected on LB medium containing 50 mg/L rifampicin and 100 mg/L carbenicillin. Selected clones are used to infect roots of *Arabidopsis* hybrid plants (described in (F) 25 above) using the root transformation protocol of Valvekens et al. (1988, PNAS 85, 5536-5540) except that shoot and root inducing media contain hygromycin (10 mg/L) instead of kanamycin.

Plants regenerated from roots of hybrid plants are genetic clones of root donating 30 plants and therefore are again genetic hybrids of two *Arabidopsis* subspecies described in (F). However, in contrast to the root donating plants, the regenerated hybrid plants also contain the introduced transgene and the co-introduced hygromycin resistance gene with the latter allowing these plants to grow on hygromycin containing culture medium.

Hygromycin resistant plants are then allowed to enter the reproductive phase and to 35 produce gametes by meiotic divisions of microspore and megasporangium mothercells. At meiosis, the *DMC1* promoter is activated and can direct the expression of antisense genes described in (B) and (C) above, leading to decreased *MSH6* and/or *MSH3* gene

expression. This in turn depletes the gamete mothercells of *MSH6* and/or *MSH3* protein, thus causing alteration of MMR during meiotic divisions and increasing the recombination frequency between homeologous chromosomes.

Transgenic plants are then allowed to self-fertilise and to produce seeds. These 5 seeds (F2 seeds with respect to hybrid production), and the plants derived therefrom, carry the homeologous recombination events which can be identified by using the visual and molecular recombination markers described in (E) above.

In case of homeologous recombination between chromosomes of *Ler* and C24(*chlorina3A:Ac, GUS*), the analysis concentrates on chromosome 2 by selecting plants 10 showing the visual phenotypic marker *chlorina*. This marker thus serves as a reference point as it indicates that respective chromosomes 2 originate from C24. Other markers, such as *GUS* or molecular markers, on chromosome 2 may then be used to identify chromosomal regions which are derived from the *Ler* chromosome as a result of homeologous recombination. F2 plants of control transformants not expressing the 15 antisense gene(s) can be analysed in parallel and the results can be used for comparison to homeologous recombination results obtained in antisense plants.

(ii) Transformation of C24 wildtype, hybrid plant production and analysis of homeologous meiotic recombination

Introduction of MMR altering genes into wildtype C24 is done using the root 20 transformation protocol as described in G(i) for transformation of hybrid plants. Transformed plants are selected by resistance to either 10 mg/L hygromycin (in case of transformation with T-DNA's derived from pNOS-Hyg-SCV) or to 50 mg/L kanamycin (in case of transformation with T-DNA's derived from pBIN19).

Transgenic plants are then allowed to self-fertilise and to produce seeds (T1 seeds). 25 Segregation of the antibiotic resistance gene in the T1 population then indicates the number of transgene loci. Lines with a single transgene locus (indicated by a 3:1 ratio of resistant:sensitive plants) are selected and are bred to homozygosity. This is done by collecting selfed seeds (T2) from T1 plants and subsequent testing of at least four independent T2 seed populations for segregation of the antibiotic resistance gene. T2 30 populations which do not segregate the antibiotic resistance gene are assumed to be homozygous for both the resistance gene and the linked MMR altering gene.

C24 plants homozygous for the MMR altering gene are then crossed to *Ler* lines 35 homozygous for recessive visual markers (see E(ii)) to produce C24/*Ler* hybrid plants as described in (F). These F1 hybrids are then allowed to enter the reproductive phase and to produce gametes by meiotic division of microspore and megasporangium mothercells. At meiosis, the *DMC 1* promoter is activated and can direct the expression of antisense or sense genes described in (B), (C) and (D) above, leading to decreased *MSH6* and/or *MSH3* gene expression. This in turn depletes the gamete mothercells of *MSH6* and/or *MSH3*

protein, thus causing alteration of MMR during meiotic divisions and increasing the recombination frequency between the homeologous chromosomes of *C24* and *Ler*. Recombination events are then scored in the F2 generation.

For recombination analysis, the hybrid plants are allowed to self-fertilise and to produce F2 seeds. F2 plants are then preselected for a first visual marker. Since this marker is recessive, its visual presence indicates homozygosity for *Ler* DNA at the relevant locus. Those F2 plants which show this first visual marker are then analysed for the presence or absence of a second visual marker which in the *Ler* parent is closely linked to the first marker. Absence of the second visual marker indicates recombination between the relevant *C24* and *Ler* chromosomes between the first and second marker. The frequency of recombination in transgenic hybrids is compared to the recombination frequency in control hybrids not expressing the MMR altering gene.

Examples of recombination analysis are the following.

The *Ler* line NW22(*dis1*, *ga4*, *th1*) is used for crosses to transformed *C24*.

15 F2 plants are preselected first for thiamine requirement (*th1*) and then are further analysed for re-appearance of wildtype height (loss of *ga4*) and/or re-appearance of normal trichomes (loss of *dis1*) as a result of recombination.

The *Ler* line NW10(*tz-201*, *cer3*) is used for crosses to transformed *C24*.

F2 plants are then preselected first for thiazole requirement (*tz*) and then are further 20 analysed for re-appearance of normal, i.e. non-shiny stems (loss of *cer3*) as a result of recombination.

The *Ler* line NW134 (*trg*, *ga3*) is used for crosses to transformed *C24*. F2 plants are first preselected for dwarfish appearance (*ga3*) and are then analysed for re-appearance of trichomes (loss of *trg*) as a result of recombination.

25 *Ler* plants homozygous for *abi3-1* and *gll-1* are used for crosses to transformed *C24*. F2 plants are first preselected for their ability to germinate in the presence of abscisic acid and are then analysed for re-appearance of trichomes on the leaves (loss of *gll-1*) as a result of recombination.

In the case of homeologous recombination between transformed *C24* and the *Ler* line 30 NW1 (*an-1*, *py-1*, *gll-1*, *cer2-1*, *ms1-1*), recombination analysis is similar the one described above, except that the second marker is not a visual marker but has to be a molecular marker. This is because the *Ler* parent carries only one visual marker per chromosome.

**TABLE 1: SSLP Markers in *Arabidopsis thaliana* Subspecies**

Chromosome	RI Map Position	PCR Primer Pair	Primer Sequence	T <sub>m</sub> [°C]	length/COL	length/LER	length/C24
I	2.3	ATEAT1 F	GCCACTGGTGAATGATATG	57.8	172	162	162
		ATEAT1 R	CGAACAGCCAACATTAAATTCCC	58.2			
I	9.3	NGA63 F	AACCCAAGGCCACAGAAGCG	57.3	111	89	120
		NGA63 R	ACCCAAGTGATGCCACC	59.6			
I	40.1	NGA248 F	TACCGAACCAAAAACACAAAGG	56.1	143	129	no amplifc.
		NGA248 R	TCTGTATCTCGGTGAATTCTCC	58.2			
I	81.2	NGA128 F	GGTCTGTTGATGTCGTAAGTCC	60.1	180	190	no amplifc.
		NGA128 R	ATCTTGAACCTTTAGGGAGGG	58.2			
I	81.2	NGA280 F	CTGATCTCACGGACAATAGTGC	60.1	105	85	85
		NGA280 R	GGCTCCATAAAAAGTGCAAC	57.8			
I	111.4	NGA111 F	CTCCAGTTGAAAGCTAAAGGG	60	128	162	170
		NGA111 R	TGTTTTTAGGACAATGGCG	70			
II	ca. 7.5	NGA168 F	CCTTCACATCCAAAACCCAC	57.8	213	217	208
		NGA168 R	GCACATACCCACAAACAGAA	57.8			

II	ca. 48	NGA1126L	CGCTACGCTTTGGTAAAG	57.8	191	199	196
		NGA1126R	GCACAGTCACAAGTCACAACC	59.9			
II	62.2	NGA361L	AAAGAGATGAGAATTGGAC	51.7	114	120	114
		NGA361R	ACATATCAATATAAAGTAGCC	49.5			
II	73	NGA168 F	TGTTCTACTGCACTGCCG	59.6	151	135	135
		NGA168 R	GAGGACATGTATAGGAGGCTG	61.9			
II	ca. 77	AthBIO2 L	TGACCTCCCTCTCCATGGAG	59.9	141	209	139
		AthBIO2 R	TTAACAGAAACCCAAAGCTTTC	54.5			
II	ca. 83	AthUBIQUE L	AGGCCAATGTCCATTTCATTG	54.1	146	148	148
		AthUBIQUE R	ACGACATGGCAGATTCTCC	57.8			
III	3.4	NGA172 F	AGCTGCTTCCCTATAGGGTCC	60	162	136	140
		NGA172 R	CATCCGAATGCCATTGTT	55.4			
III	12.8	NGA126 F	AAAAAAACGCTACTTCGTGG	56.1	119	147	no amplific.
		NGA126 R	CAAGAGCAATATCAAGAGCAGC	58.2			
III	17.5	NGA162 F	CATGCAATTGCACTGTGAGG	55.8	107	89	no amplific.
		NGA162 R	CTCTGTCACTCTTCCTCTGC	60.1			

III	81.8	NGA6 F	TCGATTCTTCCCTCTTCAC	56.1	143	123	143
		NGA6 R	ATGGAGAAGCTTACACTGATC	56.1			
IV	19.8	NGA12 F	AATGTTGTCTCCCCCTCCTC	59.9	247	234	220
		NGA12 R	TGATGCTCTCTGAAACAAGAGC	58.2			
IV	24.1	NGA8 F	GAGGGCAAATCTTTATTTCGG	56.1	154	198	190
		NGA8 R	TGGCTTTCGTTATAAACATCC	54.5			
IV	102	NGA1107 L	GCGAAAAAACAAAAAAATCCA	50.2	150	140	140
		NGA1107 R	CGACGAATCGACAGAATTAGG	58			
V	11.8	NGA225 F	GAAATCCAAATCCCAGAGAGG	58	119	189	119
		NGA225 R	TCTCCCCACTAGTTTGTC	60.1			
V	16.7	NGA249 F	TACCGTCAATTTCATCGCC	55.4	125	115	115
		NGA249 R	GGATCCCTAACTGTAAAATCCC	58.2			
V	19.9	CA72 F	ATCCCCAGTAACCAACACACA	56.3	124	110	110
		CA72 R	CCCAGTCTAACCGACCCAC	61.9			
V	20	NGA151 F	GTTTTGGGAAGTTTGTGG	55.8	150	120	130
		NGA151 R	CAGTCTAAAGGGAGAGTATGATG	58.6			

V	24	NGA106 F	GTATGGAGTTCAGGGCACG	60.1	157	123	130
V		NGA106 R	TGCCCATTTGTTCTCTCT	55.8			
V	37.8	NGA139 F	AGAGCTACCAAGATCCGATGG	59.9	174	132	132
V		NGA139 R	GGTTTCGTTCACTATCCAGG	55.8			
V	50	NGA76 F	GGAGAAAATGTCACTCTCCACC	60.1	231	>250	300
V		NGA76 R	AGGCATGGAGACATTACG	57.8			
V	61.1	ATHSO191 L	CTCCACCAATCATGCAAATG	55.8	148	156	146
V		ATHSO191 R	TGATGTTGATGGAGATGGTCA	53.7			
V	81.7	NGA129 F	TCAGGAGGAACAAAGTGAGGG	60.1	177	179	172
V		NGA129 R	CACACTGAAGATGGCTTGAGG	60.1			

## CLAIMS

1. An isolated and purified DNA molecule comprising a polynucleotide sequence encoding a polypeptide functionally involved in the DNA mismatch repair system of a plant.
- 5 2. A DNA molecule according to claim 1 wherein said polypeptide is homologous to a mismatch repair polypeptide of a yeast or of a human.
3. A DNA molecule according to claim 1 wherein said polypeptide is homologous to AtMSH3 (SEQ ID NO: 19) or to AtMSH6 (SEQ ID NO: 31).
4. An isolated and purified polypeptide functionally involved in the DNA mismatch repair system of a plant.
- 10 5. A polypeptide according to claim 4 which is homologous to a mismatch repair polypeptide of a yeast or of a human.
6. An isolated and purified polypeptide selected from the group consisting of a polypeptide encoded by the gene *AtMSH3* (SEQ ID NO: 18), a polypeptide encoded by the gene *AtMSH6* (SEQ ID NO:30), polypeptides homologous to a polypeptide encoded by the gene *AtMSH3* (SEQ ID NO: 18) and polypeptides homologous to a polypeptide encoded by the gene *AtMSH6* (SEQ ID NO:30).
7. An isolated and purified DNA molecule comprising a polynucleotide sequence selected from the group consisting of (i) a sequence encoding a polynucleotide which is capable of interfering with the expression of a plant polynucleotide sequence encoding a polypeptide which is homologous to a mismatch repair polypeptide of a yeast or of a human and thereby disabling said plant polynucleotide sequence; and (ii) a sequence encoding a polypeptide capable of disrupting the DNA mismatch repair system of a plant.
- 20 8. A DNA molecule according to claim 7 comprising a polynucleotide sequence encoding a polynucleotide capable of interfering with the expression of a plant polynucleotide sequence encoding a polypeptide which is homologous to a mismatch repair polypeptide of a yeast or of a human and thereby disabling said plant polynucleotide sequence.
9. A DNA molecule according to claim 8 wherein said polynucleotide is capable 30 of interfering with the expression of a plant polynucleotide sequence is a sense polynucleotide, an antisense polynucleotide or a ribozyme.
10. A DNA molecule according to claim 7 comprising a polynucleotide sequence encoding a polypeptide capable of disrupting the DNA mismatch repair system of a plant.

11. A DNA molecule according to claim 10 wherein said polypeptide is homologous to AtMSH3 (SEQ ID NO: 19) or to AtMSH6 (SEQ ID NO: 31).

12. A DNA molecule according to claim 10 further comprising a regulation element capable of causing overexpression of said polypeptide in a cell of said plant.

5 13. A chimeric gene comprising:

a DNA sequence selected from the group consisting of (i) a sequence encoding a polynucleotide capable of interfering with the expression of a plant polynucleotide sequence encoding a polypeptide which is homologous to a mismatch repair polypeptide of a yeast or of a human and thereby disabling said plant polynucleotide sequence, and (ii) a 10 sequence encoding a polypeptide capable of disrupting the DNA mismatch repair system of a plant; and

at least one regulation element capable of functioning in a plant cell.

14. A chimeric gene according to claim 13 wherein said regulation element is selected from constitutive, inducible, tissue type specific and cell type specific promoters.

15 15. A chimeric gene according to claim 13 comprising a DNA sequence encoding a polypeptide capable of disrupting the DNA mismatch repair system of a plant, wherein said regulation element is capable of causing overexpression of said polypeptide in a cell of said plant.

16. A chimeric gene according to claim 13 wherein said regulation element is 20 selected from the group consisting of 35S, NOS, PR1a, AoPR1 and DMC1.

17. A plasmid or vector comprising a chimeric gene according to any one of claims 13-16.

18. A plant cell stably transformed, transfected or electroporated with a plasmid or vector according to claim 17.

25 19. A plant comprising a cell according to claim 18.

20. A plant according to claim 19 selected from plants of the families *Brassicaceae*, *Poaceae*, *Solanaceae*, *Asteraceae*, *Malvaceae*, *Fabaceae*, *Linaceae*, *Canabinaceae*, *Dauaceae* and *Cucurbitaceae*.

21. A process for at least partially inactivating a DNA mismatch repair system of a 30 plant cell, comprising transforming or transfecting said plant cell with a DNA molecule according to any one of claims 1-3 or 7-12 and causing said DNA sequence to express said polynucleotide or said polypeptide.

22. A process for at least partially inactivating a DNA mismatch repair system of a plant cell, comprising transforming or transfecting said plant cell with a chimeric gene

according to any one of claims 13-16 and causing said DNA sequence to express said polynucleotide or said polypeptide.

23. A process for at least partially inactivating a DNA mismatch repair system of a plant cell, comprising transforming or transfecting said plant cell with a plasmid or vector according to claim 17 and causing said DNA sequence to express said polynucleotide or said polypeptide.

24. A process for increasing genetic variation in a plant comprising obtaining a hybrid plant from a first plant and a second plant, or cells thereof, said first and second plants being genetically different; altering the mismatch repair system in said hybrid plant; permitting said hybrid plant to self-fertilise and produce offspring plants; and screening said offspring plants for plants in which homeologous recombination has occurred.

25. A process according to claim 24 wherein a first gene is incapacitated in said first plant, a second gene is incapacitated in said second plant, and said first and second genes are incapacitated in said hybrid plant thereby altering the mismatch repair system of said hybrid plant.

25. A process according to claim 25 wherein said incapacitation of the mismatch repair system of said hybrid plant is reversible.

26. A process according to claim 24 wherein a new genetic linkage of a desired characteristic trait or of a gene which contributes to a desired characteristic trait is observable in at least one of said offspring plants.

27. A process for obtaining a plant having a desired characteristic, comprising altering the mismatch repair system in a plant, cell or plurality of cells of a plant which does not have said desired characteristic, permitting mutations to persist in said cells to produce mutated plant cells, deriving plants from said mutated plant cells, and screening said plants for a plant having said desired characteristic.

28. A process according to claim 27 wherein said step of altering the mismatch repair system comprises introducing into said hybrid plant, plant, cell or cells a chimeric gene according to claim 13 and permitting the chimeric gene to express a polynucleotide which is capable of interfering with the expression of a plant polynucleotide sequence in a mismatch repair gene of the hybrid plant, plant, cell or cells, or a polypeptide capable of disrupting the DNA mismatch repair system of the hybrid plant, cell or cells.

29. A process according to claim 28 comprising inactivating an MSH3 gene and/or an MSH6 gene of said plant.

30. A process according to claim 28 comprising inactivating an MSH3 gene and an MSH6 gene of said plant.

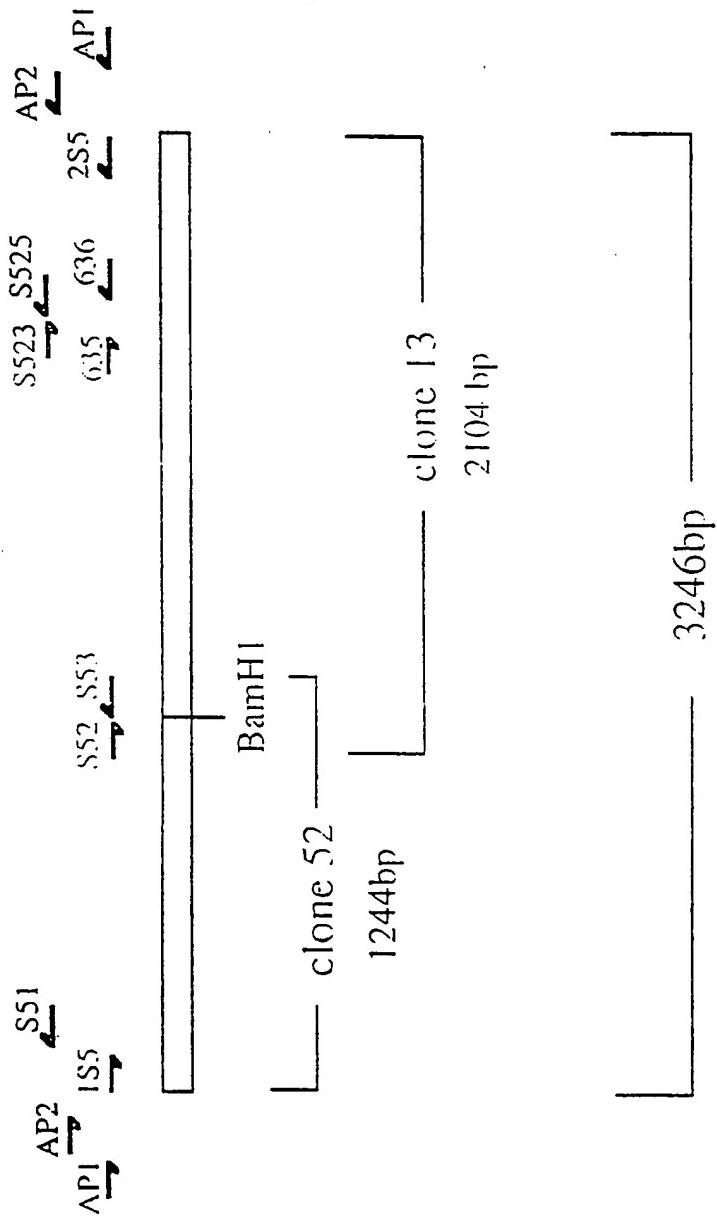
31. A process according to claim 27 comprising at least partially inactivating the mismatch repair system of said plant in a predetermined cell type or in a predetermined tissue of said plant.

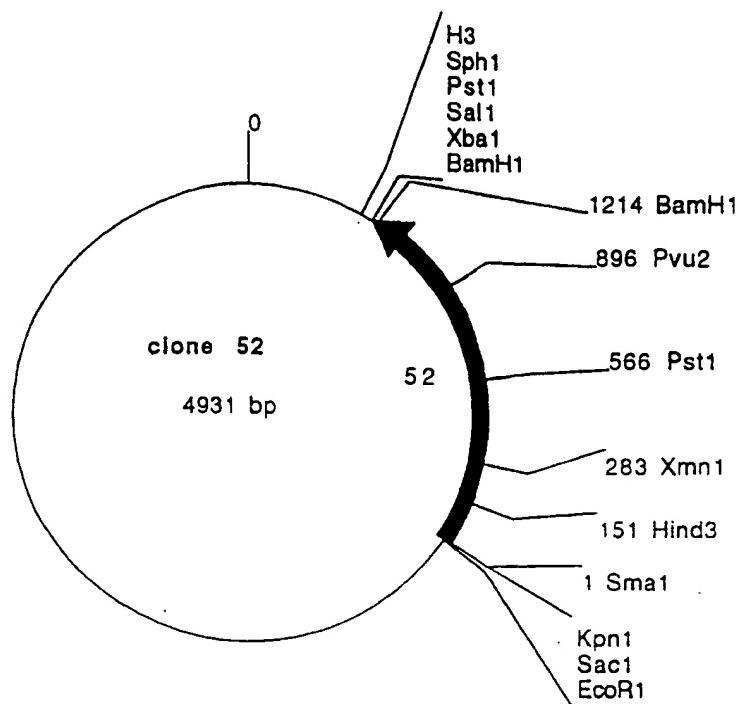
32. A process according to claim 31 further comprising restoring mismatch repair in said cell type or said tissue.

33. An oligonucleotide capable of hybridising at 45°C under standard PCR conditions to a DNA molecule according to claim 1 with the proviso that said oligonucleotide is other than SEQ ID NO:1 or SEQ ID NO:2.

34. An oligonucleotide capable of hybridising at 45°C under standard PCR conditions to the DNA of SEQ ID NO: 18 with the proviso that said oligonucleotide is other than SEQ ID NO:1 or SEQ ID NO:2.

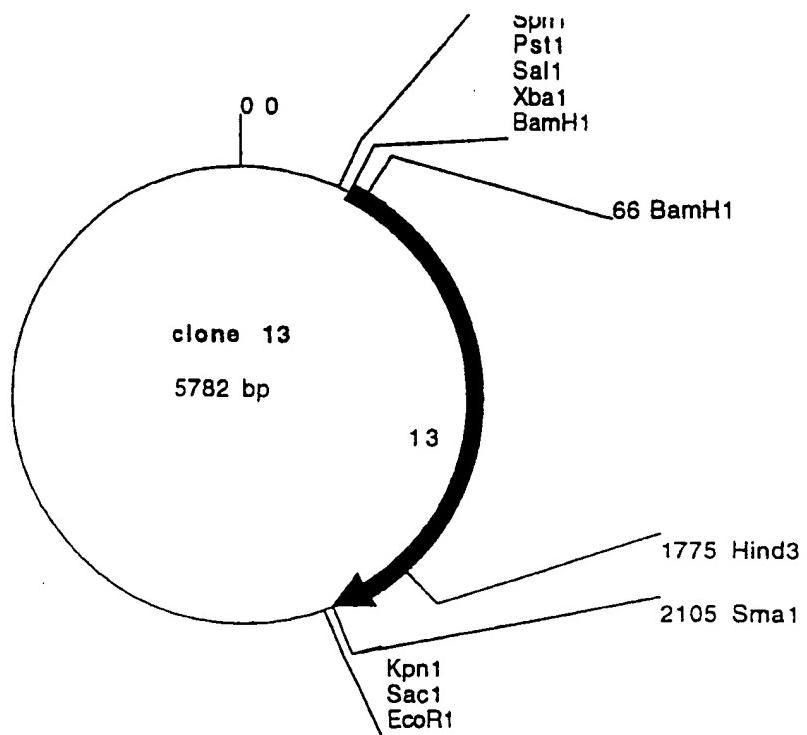
35. An oligonucleotide capable of hybridising at 45°C under standard PCR conditions to the DNA of SEQ ID NO:30 with the proviso that said oligonucleotide is other than SEQ ID NO:1 or SEQ ID NO:2.

**Figure 1**



**Figure 2**

**Comments/References:** 52= 3' side of S5 (AtMSH3) 1244bp in pUC18/Sma1



**Figure 3**

**Comments/References:** 13 = 3' side of S5 (AtMSH3) 2104bp in pUC18/Sma1

1	CCTAAGAAAGCGGGAAATTGGCAACCCRAAGTGGCCATAGGCCACGACCTTCATTCTCTAAACGGAGGA	80
81	GATTACGAATAAGCAATT ATG GGC AAG CAA AAG CAG CAG ACG ATT TCT CGT TTC GCT CCC	144
1	M G K Q K Q Q T I S R F F A P	15
145	AAA CCC AAA TCC CCG ACT CAC GAA CCG AAT CCG GTA GCC GAA TCA TCA ACA CCG CCG CCC	204
16	K P K S P T H E P N P V A E S S T P P	35
205	AAG ATA TCC GCC ACT GTA TCC TCC CCT CCT CTC TCC AAG CGT AAG CTT CTC TCC GAC CAC CTC	264
36	K I S A T V S F S P S K R K L S D H L	55
265	GCC GCG TCA CCC AAA AAG CCT AAA CTT CCT CAC ACT CAA AAC CCA GTA CCC GAT	324
56	A A S P K P K L S P H T Q N P V P D	75
325	CCC AAT TTA CAC CAA AGA TTT CTC CAG AGA TTT CTG GAA CCC TCG CCG GAG GAA TAT GTT	384
76	P N L H Q R F L Q R F L E P S P E Y V	95
385	CCC GAA ACC TCA TCA TCG AGG AAA TAC ACA CCA TTG GAA CAA GTG GTG GAG CTA AAG	444
96	P E T S S R K Y T P L E Q Q V E L K	115
445	AGC AAG TAC CCA GAT GTG GTT TTG ATG GTG GAA GTT GGT TAC AGG TAC AGA TTC TTC GGA	504
116	S K Y P D V V L M V E V G Y R F F G	135
505	GAA GAC GCG GAG ATC GCA GCA CGC GTG TTG GGT ATT TAC GCT CAT ATG GAT CAC AAT TTC	564
136	E D A E I A A R V L G I Y A H M D H N F	155
565	ATG ACG GCG ACT GTG CCA ACA TTT CGA TTG AAT TTC CAT GTG AGA AGA CTG GTG AAT GCA	624
156	M T A S V P T F R L N F H V R L V N A	175
625	GGA TAC AAG ATT GGT GTA GTG AAG CAG ACT GAA GCC ATT AAG TCC CAT GGT GCA	684
176	G Y K I G V V K Q T E T A A I K S H G A	195
665	AAC CGG ACC GGC CCT TTT TTC CGG GCA CTG TCG GCG TTG TAT ACC AAA GCC ACG CTT GAA	744
196	N R T G P F F R G L S A L Y T K A T L E	215
745	GCG GCT GAG GAT ATA AGT GGT GGT TGT GGT GAA GAA GGT TTT GGT TCA CAG AGT AAT	804
216	A A E D I S G G C G E E G F G S Q S N	235
805	TTC TTG GTT TGT GTG GAT GAG AGA GGT AAG TCG GAG ACA TTA GGC TGT GGT ATT GAA	864
236	F L V C V V D E R V K S E T L G C G I E	255
865	ATG AGT TTT GAT GTT AGA GTC GGT GTT GGC GGT GAA ATT TCG ACA GGT GAA GTT GTT	924
256	M S F D V R V G V V G V E I S T G E V V	275

Figure 4

925	TAT	GAA	GAG	TTC	AAT	GAT	AAT	TTC	ATG	AGA	AGT	GGAA	TTA	GAG	GCT	GTG	ATT	TTG	AGC	TTG	984
276	Y	E	E	F	N	D	N	F	M	R	S	G	L	E	A	V	I	L	S	L	295
985	TCA	CCA	GCT	GAG	CTG	TTG	CTT	GGC	CAG	CCT	CTT	TCA	CAA	CAA	ACT	GAG	AAG	TTT	TTG	GTG	1044
296	S	P	A	E	L	L	L	G	Q	P	L	S	Q	Q	T	E	K	F	L	V	315
1045	GCA	CAT	GCT	GGG	CCT	ACC	TCA	AAC	GTT	CGA	GTG	GAA	CGT	GCC	TCA	CTG	GAT	TGT	TTC	AGC	1104
316	A	M	A	G	P	T	S	N	V	R	V	E	R	A	S	L	D	C	F	S	335
1105	AAT	GGT	AAT	GCA	GTA	GAT	GAG	GTT	ATT	TCA	TTA	TGT	GAA	AAA	ATC	AGC	GCA	GGT	AAC	TTA	1164
336	N	G	N	A	V	D	E	V	I	S	L	C	E	K	I	S	A	G	N	L	355
1165	GAA	GAT	GAT	AAA	GAA	ATG	AAG	CTG	GAG	GCT	GAA	AAA	GGA	ATG	TCT	TGC	TTG	ACA	GTT	1224	
356	E	D	D	K	E	M	K	L	E	A	A	E	K	G	M	S	C	L	T	V	375
1225	CAT	ACA	ATT	ATG	AAC	ATG	CCA	CAT	CTG	ACT	GTT	CAA	GCC	CTC	GCC	CTA	ACG	TTT	TGC	CAT	1284
376	H	T	I	M	N	M	P	H	L	T	V	Q	A	L	A	T	F	C	H		395
1285	CTC	AAA	CAG	TTT	GGG	TTT	GAA	AGG	ATC	CTT	TAC	CAA	GGG	GCC	TCA	TTT	CGC	TCT	TTG	TCA	1344
396	L	K	Q	F	G	F	E	R	I	L	Y	Q	G	A	S	F	R	S	L	S	415
1345	AGT	AAC	ACA	GAG	ATG	ACT	CTC	TCA	GCC	ATC	ACT	CTG	CAA	CAG	TTG	GAG	GTT	GTG	AAA	AAT	1404
416	S	N	T	E	M	T	L	S	A	N	T	L	Q	Q	L	E	V	V	K	N	435
1405	AAT	TCA	GAT	GGA	TCG	GAA	TCT	GGC	TCC	TTA	TTC	CAT	AAT	ATG	AAT	CAC	ACA	CTT	ACA	GTA	1464
436	N	S	D	G	S	E	S	G	S	L	F	H	N	M	N	H	T	L	T	V	455
1465	TAT	GCT	TCC	AGG	CTT	CTT	AGA	CAC	TGG	GTG	ACT	CAT	CCT	CTA	TGC	GAT	AGA	AAT	TTG	ATA	1524
456	Y	G	S	R	L	L	R	H	W	V	T	H	P	L	C	D	R	N	L	I	475
1525	TCT	GCT	CGG	CTT	GAT	GCT	GGT	TCT	GAG	ATT	TCT	GCT	TGC	ATG	GGA	TCT	CAT	AGT	TCT	TCC	1584
476	S	A	R	L	D	A	V	S	E	I	S	A	C	M	G	S	H	S	S	495	
1585	CAG	CTC	AGC	AGT	GAG	TTG	GTT	GAA	GAA	GGT	TCT	GAG	AGA	GCA	ATT	GTA	TCA	CCT	GAG	TTT	1644
496	Q	L	S	S	E	L	V	E	E	G	S	E	R	A	I	V	S	P	E	F	515
1645	TAT	CTC	GTG	CTC	TCC	TCA	GTG	TTC	ACA	GCT	ATG	TCT	AGA	TCA	TCT	GAT	ATT	CAA	CGT	GGA	1704
516	Y	L	V	L	S	S	V	L	T	A	M	S	R	S	S	D	I	Q	R	G	535
1705	ATA	ACA	AGA	ATC	TTT	CAT	CGG	ACT	GCT	AAA	GCC	ACA	GAG	TTC	ATT	GCA	GTT	ATG	GAA	GCT	1764
536	I	T	R	I	F	H	R	T	A	K	A	T	E	F	I	A	V	M	E	A	555
1765	ATT	TTA	CTT	GCG	GGG	AAG	CAA	ATT	CAG	CGG	CTT	GGC	ATA	AAG	CAA	GAC	TCT	GAA	ATG	AGG	1824
556	I	L	L	A	G	K	Q	I	R	L	G	I	K	Q	D	S	E	M	R	575	

Figure 4 (Continued)

1825	AGT	ATG	CAA	TCT	GCA	ACT	GTG	CGA	TCT	ACT	CCT	TTC	TG	AGA	AAA	TTG	ATT	TCT	GTT	ATT	TCA	1884
576	S	M	Q	S	A	T	V	R	S	T	L	L	R	K	L	I	S	V	I	S	595	
1885	TCC	CCT	GTT	GTG	GTT	GAC	AAT	GCC	AAA	CTT	CTC	TCT	GCC	CTA	AAT	AAG	GAA	GCG	GCT	1944		
596	S	P	V	V	D	N	A	G	K	L	L	L	S	A	L	N	K	E	A	A	615	
1945	GTT	CGA	GGT	GAC	TTG	CTG	GAC	ATA	CTA	ATC	ACT	TCC	AGC	GAC	CAA	TTT	CCT	GAG	CTT	GCT	2004	
616	V	R	G	D	L	L	D	I	L	I	T	S	S	D	D	Q	F	P	E	L	A	635
2005	GAA	GCT	CGC	CAA	GCA	GCA	GTC	GT	TTA	GTC	ATC	AGG	GAA	AAG	CTG	GAT	TCC	TCG	ATA	GCT	TCA	2064
636	E	A	R	Q	A	V	I	V	I	R	E	K	L	D	S	S	I	A	S	F	655	
2065	CGC	AAG	AAG	CTC	GCT	AT	CGA	AA	T	CGA	TTG	GAA	TTG	CNA	GTT	G	G	I	T	H	L	2124
656	R	K	K	L	A	I	R	N	L	E	F	L	Q	V	S	G	I	T	H	L	675	
2125	ATA	GAG	CTG	CCC	GTT	GAT	TCC	AAG	GTC	CCT	ATG	AA	T	TGG	GTG	AAA	GTA	AAT	AGC	ACC	AAG	2184
676	I	E	L	P	V	D	S	K	V	P	H	N	W	V	K	V	N	S	T	K	695	
2185	AAG	ACT	ATT	CGA	TAT	CAT	CCC	CCA	GAA	ATA	GTA	GCT	GCG	TTC	GAT	GAG	CTA	GCT	CTA	GCA	2244	
696	K	T	I	R	Y	H	P	P	E	I	V	A	G	L	D	E	L	A	L	A	715	
2245	ACT	GAA	CAT	CTT	GCC	ATT	GTG	AAC	CGA	GCT	TG	TG	GAT	AGT	TTC	CTC	AAG	AGT	TTC	AGT	2304	
716	T	E	H	L	A	I	V	N	R	A	S	W	D	S	F	L	K	S	F	S	735	
2305	AGA	TAC	TAC	ACA	GAT	TTT	AAG	GCT	GCC	GTC	CTT	GCA	GCT	CTG	GAC	TGT	TG	CAC	2364			
736	R	Y	Y	T	D	F	K	A	A	V	Q	A	L	A	L	D	C	L	H	755		
2365	TCC	CTT	TCA	ACT	CTA	TCT	AGA	AAC	AAG	AA	TAT	GTC	CGT	CCC	GAG	TTT	GTG	GAT	GAC	TGT	2424	
756	S	L	S	T	L	S	R	N	K	N	Y	V	R	P	E	F	V	D	D	C	775	
2425	GAA	CCA	GTT	GAG	ATA	AAC	ATA	CAG	TCT	GCT	CAT	CCT	GTA	CTG	GAG	ACT	ATA	TTA	CAA	2484		
776	E	P	V	E	I	N	I	Q	S	G	R	H	P	V	L	E	T	I	L	Q	795	
2485	GAT	AAC	TTC	GTC	CCA	AA	T	GAC	ACA	ATT	TG	CAT	GCA	GAA	GGG	GAA	TAT	TGC	CAA	ATT	ATC	2544
796	D	N	F	V	P	N	D	T	I	L	H	A	E	G	E	Y	C	Q	I	I	815	
2545	ACC	GG	CCT	AAC	ATG	GG	GG	AAG	AGC	TGC	TAT	ATC	CGT	CAA	GT	GCT	TTA	ATT	TCC	ATA	2604	
816	T	G	P	N	M	G	G	K	S	C	Y	I	R	Q	V	A	L	I	S	I	835	
2605	ATG	GCT	CAG	GTT	GGT	TCC	TTT	GTA	CCA	GCG	TCA	TTC	GCC	AAG	CTG	CAC	GTG	CTT	GAT	GGT	2664	
836	W	A	Q	V	G	S	F	V	P	A	S	F	A	K	L	H	V	L	D	G	855	
2665	GTT	TTC	ACT	CGG	ATG	GGT	GCT	TCA	GAC	AGT	ATC	CAG	CAT	GGC	AGA	AGT	ACC	TTT	CTA	GAA	2724	
856	V	F	T	R	M	G	A	S	D	S	I	Q	H	G	R	S	T	F	L	E	875	

Figure 4 (Continued)

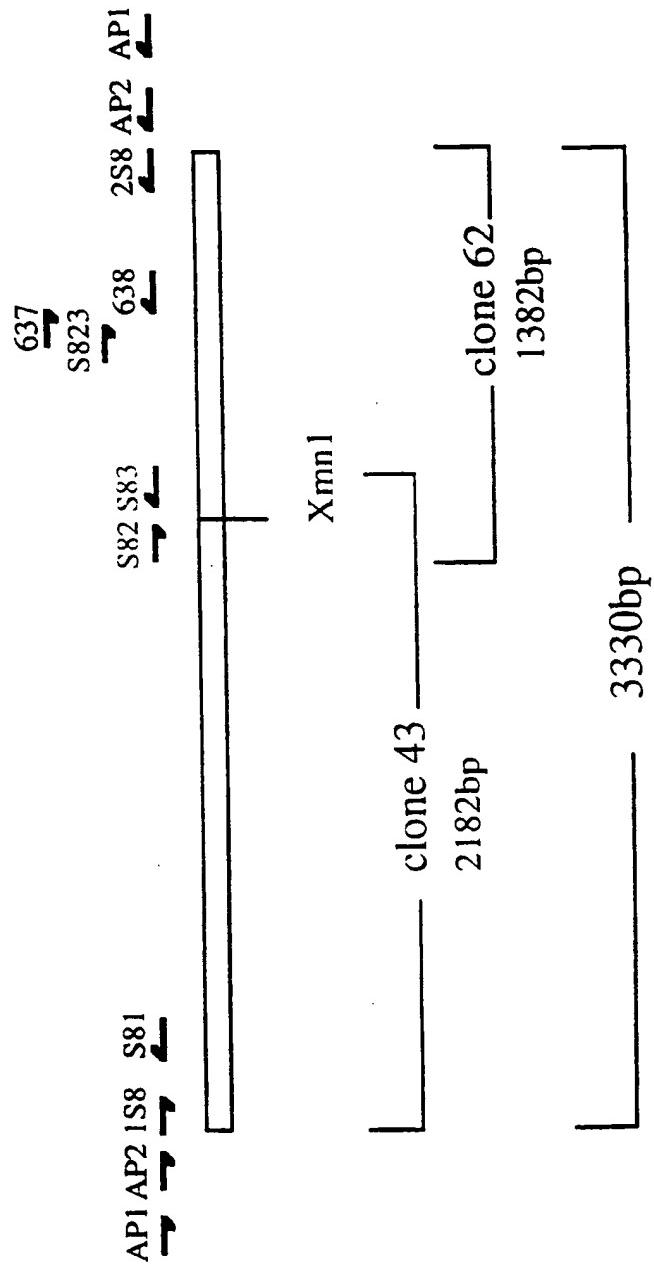
2725	GAA TTA AGT GAA GCG TCA CAC ATA ATC AGA ACC TGT TCT TCG CTC CGT TCT ATA TTA 876      E    L    S    E    A    S    H    I    I    R    T    C    S    R    S    L    V    I    L	2784      895
2785	GAT GAG CTT GGA AGA GGC ACT AGC ACA CAC GAC GGT GTA GCC ATT GCC TAT GCA ACA TTA 896      D    E    L    G    R    G    T    S    T    H    D    G    V    A    I    A    Y    A    T    L	2844      915
2845	CAG CAT CTC CTA GCA GAA AAG AGA TGT TTG GTT CTT GTC ACG CAT TAC CCT GAA ATA 916      Q    H    L    L    A    E    K    R    C    L    V    L    F    V    T    H    Y    P    E    I	2904      935
2905	GCT GAG ATC AGT AAC GGA TTC CCA GGT TCT GTT GGG ACA TAC CAT GTC TCG TAT CTG ACA 936      A    E    I    S    N    G    F    P    G    S    V    G    T    Y    H    V    S    Y    L    T	2964      955
2965	TTG CAG AAG GAT AAA GGC AGT TAT GAT CAT GAT GTG ACC TAC CTA TAT AAG CTT GTG 956      L    Q    K    D    K    G    S    Y    D    H    D    V    T    Y    L    Y    K    L    V	3024      975
3025	CGT GGT CTT TGC AGC AGG AGC TTT GGT TTT AAG GTT GCT CAG CTT GCC CAG ATA CCT CCA 976      R    G    L    C    S    R    S    F    G    F    K    V    A    Q    L    A    Q    I    P    P	3084      995
3085	TCA TGT ATA CGT CGA GCC ATT TCA ATG GCT GCA AAA TTG GAA GCT GAG GTA CGT GCA AGA 996      S    C    I    R    R    A    I    S    M    A    A    K    L    E    A    E    V    R    A    R	3144      1015
3145	GAG AGA AAT ACA CGC ATG GGA GAA CCA GAA CAT GAA GAA CCC AGA GGC GCA GAA GAA 1016      E    R    N    T    R    M    G    E    P    E    G    H    E    E    P    R    G    A    E    E	3204      1035
3205	TCT ATT TCG GCT CTA GGT GAC TTG TTT GCA GAC CTG AAA TTT GCT CTC TCT GAA GAG GAC 1036      S    I    S    A    L    G    D    L    F    A    D    L    K    F    A    L    S    E    E    D	3264      1055
3265	CCT TGG AAA GCA TTC GAG TTT TTA AAG CAT GCT TGG AAG ATT GCT GGC AAA ATC AGA CTA 1056      P    W    K    A    F    E    F    L    K    H    A    W    K    I    A    G    K    I    R    L	3324      1075
3325	AAA CCA ACT TGT TCA TTT TGA TTTAACATTAGCAACTGCCAAGGCTTAGTTGATCATCTGTTAGTTGCC 1076      K    P    T    C    S    F    *	3397      1082
3398	TACTAACCT ATG TGT ATT AGT ATA ACA AGA AAA GAG AAT TAG AGAG ATG GAT TCT ATAT CCG 1      M    C    I    S    I    T    R    K    E    N    *    M    D    S    N    P	3458      5
3459	GTG TTG CAG TAC ATC TTT TCT CCA CCC GCA TAA AAAA AAAAAA AAAAAA AAAAAA AAAAAA 6      V    L    Q    Y    I    F    S    P    P    A    *	3522      16

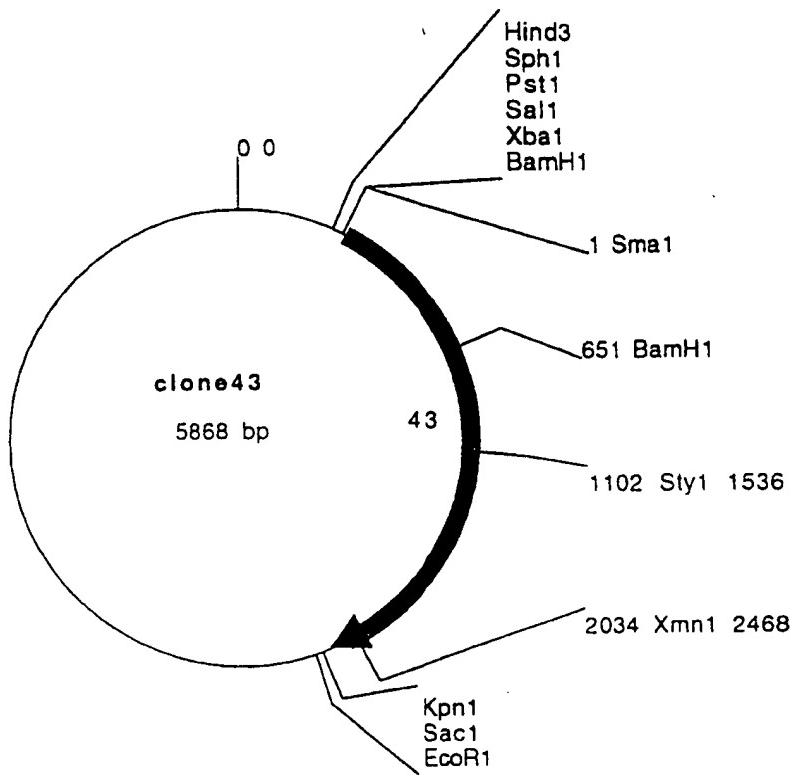
Figure 4 (Continued)

Figure 5

1 - MIGKOK-----QOTISREFAPPKPKSP-THE-PNPVYASSTPPPK-----ISAYVSFSYKHNLL  
 1 MVICGNEPKLVLRAKSSANRPNILLTIAUGOPTISRPKAVKSELTHKQEVAVNGAGSICLUDDEDNLSVASTYNTDSYKLN  
 MSH3-AC 52 SDHLLAAASPKEPKLSPSEQHPVDPUNLHQPLURFLP-----SPEEVYFETTS-S---SRYVTPLEQVVVSKSYDVEVNGY  
 MSH3-SC 96 VS SKWNSKNSWTSGCTSTPNDIDFAKKDRIMKRSDENVEEEDDEGENEEDPKKAKNSPTAKLTLPLKOKVDRKNNRDAK  
 MSH3-AC 134 EDEDEIAARVEGFIAB-----MDHN-----PNTASVPTFLNPHYARLYNAOYKIQVVAETTAIXSH-JAATGAPPRLGSLATXKA  
 MSH3-SC 191 EDADAVTWSRKEKLVPGKLTDMSNPQUCNHNQPAYCEPDVRLNVHLERLVHNLKVAVVEQAEESAKKEDPAKSSVPERKSHVPTKA  
 MSH3-AC 213 ELZAAEDISGGCGGEEFGQSNSVPLCVYDERVKSETWUCCIGIENSYUWVQVOYVISEGEVNYEF-D-PRMRSOFAVLSHSPAEV  
 MSH3-SC 266 FPGVNSTFVR-----GKR-----ILUDTNIMASRVDHQKVKAVYSLISUNMGEUVYDDEFEPBLADEKEDH  
 MSH3-AC 306 EQUOTEKFELVANAQOPTSNVVERASLDGYNGNADEVISCEKISAGNLEDKEMKLEAKKGMSCLTVHTIMMPHLTVOALALTCHLKOFG  
 MSH3-SC 368 EFLHVAKFFKD DISCPLINHKQYDLEUVQAKVMMNEKFOESPVL-----  
 MSH3-AC 401 PERILYQGASVNSLSSNTERPSA-----EQQLEVVKNNNSDGSZSGLSFHNMMHTLIVIGSRLEPQYDMLISARLDAYSEIPACMGSSSS  
 MSH3-SC 428 NEQVNLIPSIYSPASKHNLDFPSLSDP-----GCK-GSLPMLLDHTHESYGLMRERFILKPEVHQKERDACEKTCITSEINNS...  
 MSH3-AC 496 QLSSELVNEQSERAIYSPPEPVLYLSQVLTAMMSKSDIOQUTTRIPHAKATEYIAVMEALLACKQIQRLGIQDSEMM5QS-ATVMSCLRM  
 MSH3-SC 517 -----IPEELQWMLNTPDLLRFLNRIMYGTSHKEVYVYKQIITSPVDSHKWQSYLSMTPKSISDORIUKQSPLLP  
 MSH3-AC 590 ERSVISSPVVVDAGKLEPAAKRELAVERG-----DQPELAEARQAVLVIKEFQSSIASRRENQIIRMEFLQVSGITHLIEKP  
 MSH3-SC 591 EPSEBLNLSTTOLPHFETMIVSVMEKNSDQVMOPPNLNNDCSEGIIKURSESVRQLKESLAFISKYKRPYTFNADDEVDLIEVKNS  
 MSH3-AC 680 VDSKVPMNTRKVSITRUPPEIVAGLDEHALATEHAIVNKAASWDSEFLKSPGRYTTPEAVQAEDLCCTNSBTLSPFHRYVAPFVUD  
 MSH3-SC 686 QIKDLPDDKIKVNTKMKVSRITPRTOKLQKETYKDLIKESELQYKEPLNKITAETFLAKITLNAYD  
 MSH3-AC 775 CEPVEINIQSCRHAPVLETIQDNYVPNDJLMAEGYCYQIITGPNUCKXCYIRQVALQEHAQVGSPPVAPASVANHULDOVPPRGASDSDIQG  
 MSH3-SC 781 QO--ATIAKARNMPITES-HDVHYVPNDHMSIPENGKIMITGPNUCKXCYIRQVALQEHAQIGSPVPAZLISIVNHLTRIGANDDING  
 MSH3-AC 870 RSTFLELSKASHIIRTCSSRSLVILDELJGRTSTHDGVAIAYARLOHLLAEKRE-CYCFVHYPRIAETISNUPPGSYOTHVSYLTLOKOKGST  
 MSH3-SC 873 DTEPKVENDLILHILKNCWRSLLDEVGRTUDGIAISYALIKYVSELSDCPKILETHYPMLOEIKS--PLRNMHDIVEOK-TCE  
 MSH3-AC 964 CHDDEPTEKXKEVAGLCSRSYGPKVACQCAQIPSCIRRAISAAKDEAEVARENNTNGEPEQGHHEPRAQEESISAQODLSEPEXPAKEDPNK  
 MSH3-SC 963 CHMSEVYKRLKQGFLYNSKGM-NVAKLARDLIDINRAPSISERAKESIN-----EDALEE--ESSLRKRIEKSIN-----  
 MSH3-AC 1059 APEFLKHAKH-----KPKCSR-----  
 MSH3-SC 1032 -----HATDPEAKLSDIH

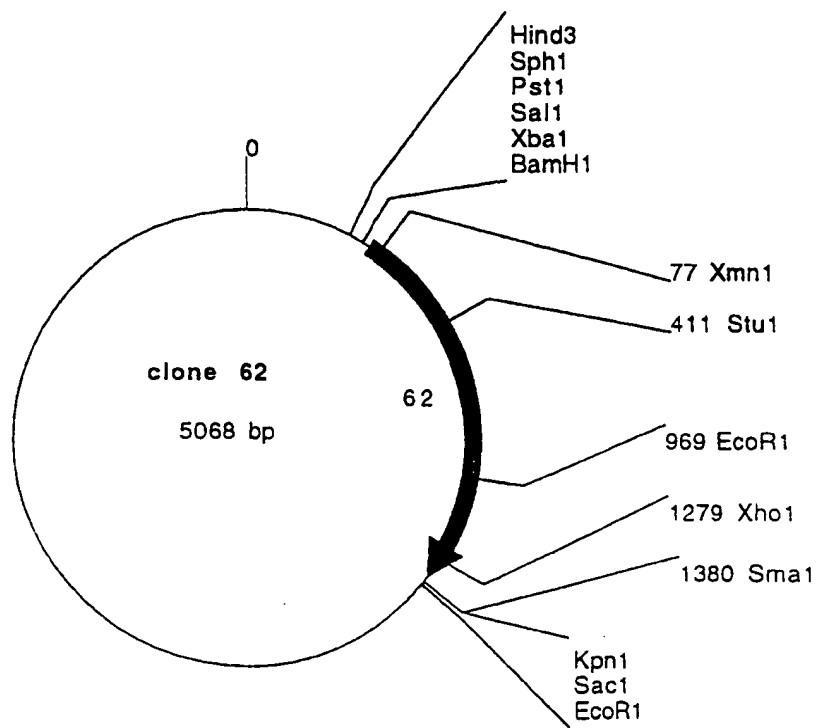
Figure 6





**Figure 7**

**Comments/References:** 43= 5' side of S8 (AtMSH6) 2182 bp in pUC18/Sma1



**Figure 8**

**Comments/References:** 62= 3' side of S8 (AtMSH6) 1379bp in pUC18/Sma1

1	AAAAGTTGAGGCCCTGAGGAGTATCGTTCGCCATTCTACTCTCACTCTCACTCTCACAAATTCCAAAAAATCTTGGGGCCAATCTTCCCCCC	153
81	TTTCGAATTCTCTCAAGCTCAAAACATCGTTCTCTCACTCTCACTCTCACAAATTCCAAAAAATG CAG CGC CAG M Q R Q	4
1		
1154	AGA TCG ATT TTG TCT TTC CAA AAA CCC ACC GCG GCG ACT ACG AAG GGT TTG GTT TCC R S I L S F F Q K P T A A T T K G L V S	213
5	GGC GAT GCT GCT AGC GGC GGG GGC AGC GGA GGA CCA CGA TTT AAT GTG AAG GAA GGG G D A A S G G G S G P R F N V R E G	273
214	GAT GCT AAA GGC GAC GCT TCT GTA CGT TTT GCT GTT TCG AAA TCT GTC GAT GAG GTT AGA D A K G D A S V R F A V S K S V D E V R	333
274	GGA AGG GAT ACT CCA CCG GAG AAG GTT CCG CGT CGT GTC CTG CCG TCT GGA TTT AAG CCG G T D T P P E K V P R R V L P S G F K P	393
45	GCT GAA TCC GCC GST GAT GCT TCG TCC TTC TCC AAT ATT ATG CAT AAG TTT GTA AAA A E S A G D A S S L F S N I M H K F V K	84
394	GTC GAT CGA GAT TGT TCT GGA GAG AGC CGA GAA GAT GTT CCG CTG AAT ATT ATG CAT AAG TTT GTA AAA 85	104
85	D R D C S G E R S R E D V V P L N D	453
454	TCA TCT CTA TGT ATG AAG GCT AAT GAT GTT ATT CCT CAA TTT CGT TCC AAT GAT GAT V D S L C M K A N D V I P Q F R S N N G K	513
105	ACT CAA GAA AGA AAC CAT GCT TTT AGT TTC AGT GGG AGA GCT GAA CTT AGA TCA GTA GAA T Q E R N H A F S G R A E L R S V E	124
514	GAT ATA GGA GTA GAT GGC GAT GTT CCT GGT CCA GAA ACA CCA GGG ATG CGT CCA CGT GCT 125	144
125	D I G V D G D V P G P E T P G M R P R A	633
574	TCT CGC TTG AAG CGA GTT CTG GAG GAT GAA ATG ACT ATT AAG GAG GAT AAG GTT CCT GTA 145	164
145	S R L K R V L E D E M T F K E D K V P V	693
634	TTG GAC TCT AAC AAA AGG CTG AAA ATG CTC CAG GAT CCG GTT TGT GGA GAG AAA GAA V N E G T K F E W L E S S R I R D A N R	184
165	GTA AAC GAA GGA ACC AAA TTT GAA TGG CTT GAG TCT TCT CGA ATC AGG GAT GCC AAT AGA 225	753
694	R R P D P D P L Y D R K T L H I P P D V F	873
185	AGA CGT CCT GAT GAT CCC CTT TAC GAT AGA AAG ACC TTA CAC ATA CCA CCT GAT GTT TTC 245	933
205	R R P D P D P L H I P P D V F	264

9

934	AAG AAA ATG TCT GCA TCA CAA AAG CAA TAT TGG AGT GTT AAG AGT GAA TAT ATG GAC ATT	993
265	K K M S A S Q K Q Y W S V K S E Y M D I	284
996	GTG CTT TTC TTT AAA GTG GGG AAA TTT TAT GAG CTG TAT GAG CTA GAT GCG GAA TTA GGT	1053
285	V L F F K V G K F Y E L Y E L D A E L G	304
1054	CAC AAG GAG CTT GAC TGG AAG ATG ACC ATG AGT GGT GTG GGA AAA TGC AGA CAG GTT GGT	11113
305	H K E L D W K M T M S G V G K C R Q V G	324
1114	ATC TCT GAA AGT GGG ATA GAT GAG GCA GTG CAA AAG CTA TTA GCT CGT GGA TAT AAA GTT	1173
325	I S E S G I D E A V Q K L A R G Y K V	344
1174	GGA CGA ATC GAG CAG CTA GAA ACA TCT GAC CAA GCA AAA GCC AGA GGT GCT ATT ACT ATA	1233
345	G R I E Q L E T S D Q A K A R G A N T I	364
1234	ATT CCA AGG AAG CTA GTT CAG GTA TTA ACT CCA TCA ACA GCA AGC GAG GGA AAC ATC GGG	1293
365	I P R K L V Q V L T P S T A S E G N I G	384
1294	CCT GAT GCC GTC CAT CTT CTT GCT ATA AAA GAG ATC AAA ATG GAG CTA CAA AAG TGT TCA	1353
385	P D A V H L L A I K E I K M E L Q K C S	404
1354	ACT GTG TAT GGA TTT GCT TTT GAC TGT GCT GCC TTG AGG TTT TGG GRT GGG TCC ATC	1413
405	T V Y G F A F V D C A L R F W V G S I	424
1414	AGC GAT GAT GCA TCA TGT GCT C'RT GGA GCG TTA TTG ATG CAG GTT TCT CCA AAG GAA	1473
425	S D D A S C A A L G A L M Q V S P K E	444
1474	GTG TTA TAT GAC AGT AAA GGG CTA TCA AGA GAA GCA CAA AAG GCT CTA AGG AAA TAT ACG	1533
445	V L Y D S K G L S R E A Q K A L R K Y T	464
1534	TTG ACA GGG TCT ACG GCG GTA CAG TTG GCT CCA GTA CCA CAA GTA ATG GGG GAT ACA GAT	1593
465	L T G S T A V Q L A P V P M G D T D	484
1594	GCT GCT GGA GRT AGA AAT ATA GAA TCT AAC GGA TAC TTT AAA GGT TCT TCT GAA TCA	1653
485	A A G V R N I I E S N G Y F K G S S E S	504
1654	TGG AAC TGT GCT GGT GAT GGT CTA AAT GAA TGT GAT GTT GCC CTT AGT GCT CTT GGA GAG	1713
505	W N C A V D G L N E C D V A L S A L G E	524
1714	CTA ATT AAT CAT CTG TCT AGG CTA AAG CTA GAA GAT GTA CTT AAG CAT GGG GAT ATT TTT	1773
525	L I N H L S R L K L E D V L K H G D I F	544
1774	CCA TAC CAA GTT TAC AGG GGT TGT CRC AGA ATT GAT GGC CAG ACG ATG GTA AAT CTT GAG	1833
545	P Y Q V Y R G C L R I D G Q T M V N L E	564

Figure 9 (Continued)

1834	ATA TTT AAC AAT AGC TGG GAT GGT CCT TCA GGG ACC TTG TAC AAA TAT CTT GAT AAC	1893
565	I F N N S C D G G P S G T L Y K Y L D N	584
1894	TGT GTT AGT CCA ACT GGT AAG CGA CTC TTA AGG AAT TGG ATC TGC CAT CCA CTC AAA GAT	1953
585	C V S P T G K R L R N W I C H P L K D	604
1954	GTA GAA AGC ATC AAT AAA CGG CTT GAT GTC GTT GAA GAA TTC ACG GCA AAC TCA GAA AGT	2013
605	V E S I N K R L D V V E F T A N S E S	624
2014	ATG CAA ATC ACT GGC CAG TAT CTC CAC AAA CTT CCA GAC TTA GAA AGA CTG CTC GGA CGC	2073
625	M Q I T G Q Y L H K L P D L E R L L G R	644
2074	ATC AAG TCT AGC GTT CGA TCA TCA GCC TCT GTG TTG CCT GCT CRT CTG GGG AAA AAA GTG	2133
645	I K S S V R S A S V I p A L L G K K V	664
2134	CTG AAA CAA CGA GTT AAA GCA TTT GGG CAA ATT GTG AAA GGG TTC AGA ACT GGA ATT GAT	2193
665	L K Q R V K A F G Q I V K G F R S G I D	684
2194	CTG TTG TTG GCT CTA CAG AAG GAA TCA AAT ATG	2253
685	L L A L Q K E S N M M S L L Y K L C K	704
2254	CTT CCT ATA TTA GTA GGA AAA AGC GGG CTA GAG TTA TTT CTT TCT CAA TTC GAA GCA GCC	2313
705	L P I L V G K S G L E L F I S Q F E A A	724
2314	ATA GAT AGC GAC TTT CCA AAT TAT CAG AAC CAA GAT GTG ACA GAT GAA AAC GCT GAA ACT	2373
725	I D S D F P N Y Q N Q D V T D E N A E T	744
2374	CTC ACA ATA CTT ATC GAA CTT TTT ATC GAA AGA GCA ACT CAA TGG TCT GAG GTC ATT CAC	2433
745	L T I L I E L F I A T Q W S E V I H	764
2434	ACC ATA AGC TGC CTA GAT GTC CTG AGA TCT TTT GCA ATC GCA GCA AGT CTC TCT GCT GGA	2493
765	T I S C L D V L R S F A I A S L S A G	784
2494	AGC ATG GCC AGG CCT GTT ATT TTT CCC GAA TCA GAA GCT ACA GAT CAG AAA ACA	2553
785	S M A R P V I F P E S E A T D Q N Q K T	804
2554	AAA GGG CCA ATA CTT AAA ATC CAA GGA CTA TGG CAT CCA TTT GCA GTT GCC GGC GAT GGT	2613
805	K G P I L K I Q G L W H P F A V A D G	824
2614	CAA TTG CCT GTT CCG AT ATG ATA CTC CTT GGC GAG GCT AGA AGC AGT GGC AGC ATT	844
825	Q L P V P N D I L L G E A R R S S G S I	864
2674	CAT CCT CGG TCA TTG TTA CTG ACG GGA CCA AAC ATG GGC GGA AAA TCA ACT CTT CTT CGT	2733
845	H P R S I L L T G P N M G G K S T L L R	864

**Figure 9** (Continued)

2734	GCA	ACA	TGT	CTG	GCC	GTT	ATC	TTT	GCC	CAA	CTT	GGC	TAC	GTG	CCG	TGT	GAG	TCT	TGC	2793	
865	A	T	C	L	A	V	I	F	A	Q	L	G	C	Y	V	P	C	E	S	C	884
2794	GAA	ATC	TCC	CTC	GTG	GAT	ACT	ATC	TTC	ACA	AGG	CTT	GGC	GCA	TCT	GAT	AGA	ATC	ATG	ACA	2853
885	E	I	S	L	V	D	T	I	F	T	R	L	G	A	S	D	R	I	M	T	904
2854	GGA	GAG	AGT	ACC	TTT	TTG	GTA	GAA	TGC	ACT	GAG	ACA	GGG	TCA	GTT	CIT	CAG	AAT	GCA	ACT	2913
905	G	E	S	T	F	L	V	E	C	T	E	T	A	S	V	L	Q	N	A	T	924
2914	CAG	GAT	TCA	CTA	GTA	ATC	CTT	GAC	GAA	CTG	GGC	AGA	GGG	ACT	AGT	ACT	TTC	GAT	GGA	TAC	2973
925	Q	D	S	L	V	I	L	D	E	L	G	R	G	T	S	T	F	D	G	Y	944
2974	GCC	ATT	GCA	TAC	TCG	GTT	TTT	CGT	CAC	CTG	GTA	GAG	AAA	GTT	CAA	TGT	CGG	ATG	CTG	CTC	3033
945	A	I	A	Y	S	V	F	R	H	L	V	E	K	V	Q	C	R	M	L	F	964
3034	GCA	ACA	CAT	TAC	CAC	CCT	CTC	ACC	AAG	GAA	TTC	GGG	TCT	CAC	CCA	CGT	GTC	ACC	TCG	AAA	3093
965	A	T	H	Y	H	P	L	T	K	E	F	A	S	H	P	R	V	T	S	K	984
3094	CAC	ATG	GCT	TGC	GCA	TTC	AAA	TCA	AGA	TCT	GAT	TAT	CAA	CCA	CGT	GGT	TGT	GAT	CAA	GAC	3153
985	H	M	A	C	A	F	K	S	R	S	D	Y	Q	P	R	G	C	D	Q	D	1004
3154	CTA	GTG	TTC	TTG	TAC	CGT	TTA	ACC	GAG	GGA	GCT	TGT	CCT	GAG	AGC	TAC	GGG	CTT	CAA	GTG	3213
1005	L	V	F	L	Y	R	L	T	E	G	A	C	P	E	S	Y	G	L	Q	V	1024
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1025	A	L	M	A	G	I	P	N	Q	V	E	T	A	S	G	A	Q	A	Q	A	1044
3274	ATG	AAG	AGA	TCA	ATT	GGG	GGA	AAC	TTC	AGG	TCA	AGT	CAG	CYA	AGA	TCT	GAG	TTC	TCA	AGT	3333
1045	M	K	R	S	I	G	E	N	F	K	S	S	E	L	R	S	E	F	S	S	1064
3334	CTG	CAT	GAA	GAC	TGG	CTC	AAG	TCA	TTG	GTG	GGT	ATT	TCT	CGA	GTC	GCC	CAC	AAC	AAT	GCC	3393
1065	L	H	E	D	W	L	K	S	L	V	G	I	S	R	V	A	H	N	N	A	1084
3394	CCC	ATT	GGC	GAA	GAT	GAC	TAC	GAC	ACT	TTG	TTT	TGC	TTA	TGG	CAT	GAG	ATC	AAA	TCC	TCT	3453
1085	P	I	G	E	D	D	Y	D	T	L	F	C	L	W	H	E	I	K	S	S	1104
3454	TAC	TGT	GTT	CCC	AAA	TAA	ATG	GCT	ATG	ACA	TAA	CACTATCTGAAGCTCGTTAAGTCTTGTGCTCTCT	*	*	*	*	*	*	*	5	
1105	Y	C	V	P	K	*	M	A	M	T	*	*	*	*	*	*	*	*	*	*	19
3522	G	ATG	TTT	ATT	CCT	CTT	AAA	AAA	TGC	TTA	TAT	ATC	AAA	TTG	TTT	CCT	CGA	TTA	AAA	3579	
1	M	F	I	P	L	K	K	C	L	Y	I	K	K	L	F	P	R	L	K	3606	
3580	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	28											
20	K	K	K	K	K	K	K	K	K	K	K	K	K	K	K	K	K	K	K	Figure 9 (Continued)	

Figure 10

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CACGTGCTCTCGTTGAAGCGAGTTCTGGAGGATGAAATGACTTTAAG	2000
GAGGATAAGGTTCTGTATTGGACTCTAACAAAAGGCTGAAAATGCTCCA	2050
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GATCCCCCTTACGATAGAAAGACCTTACACATACCACTGATGTTCAA	2200

Figure 11

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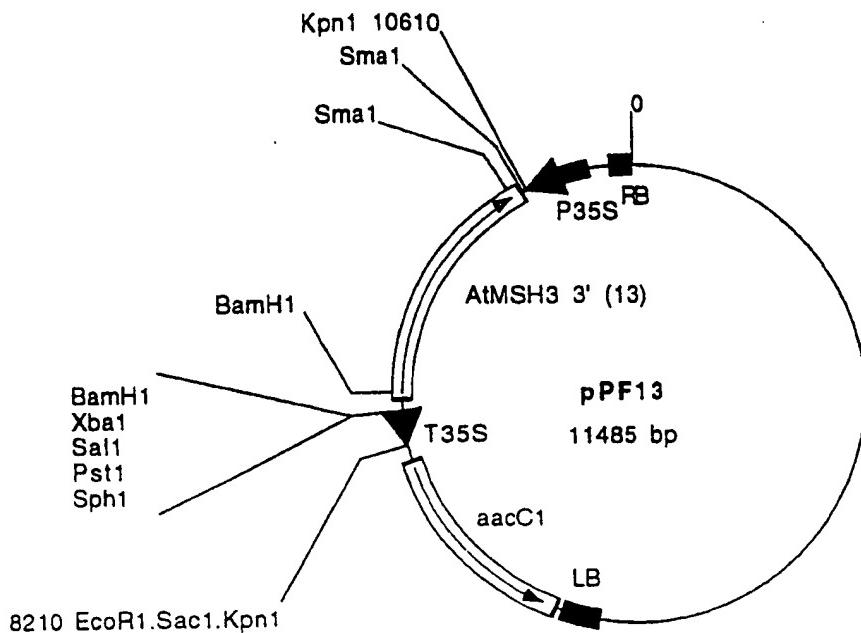
Figure 11 (Continued)

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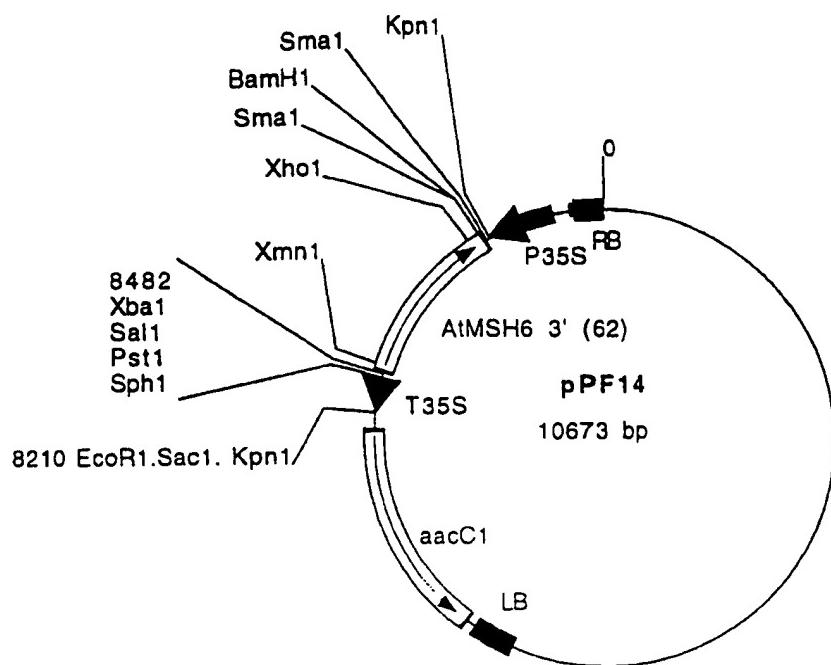
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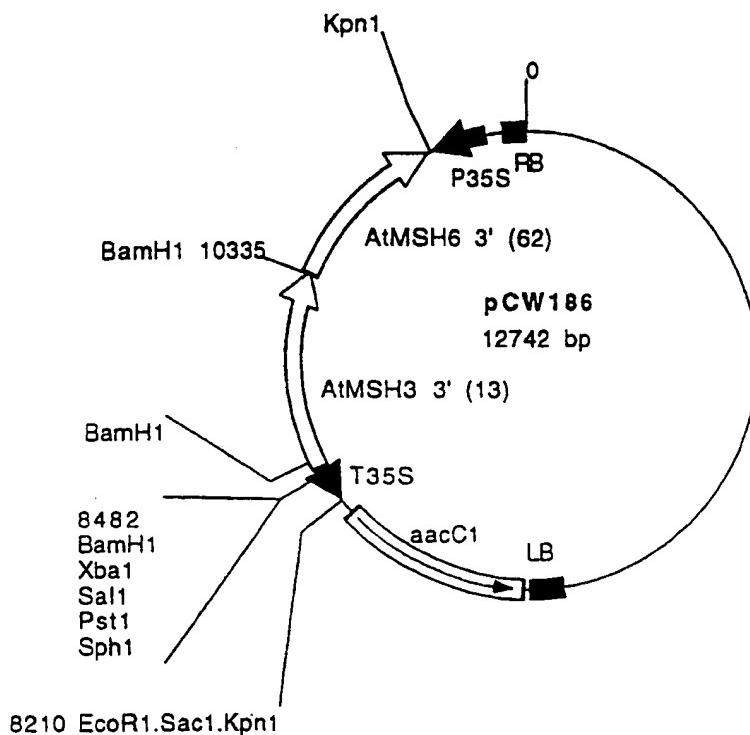
**Figure 12**

**Comments/References:** AtMSH3 3' side antisense : AtMSH3 3' (13 = 2104bp) from pUC18/13 Sal1/Sst1/T4 into pCW164 BamH1/T4 in Agrobacterium LBA4404



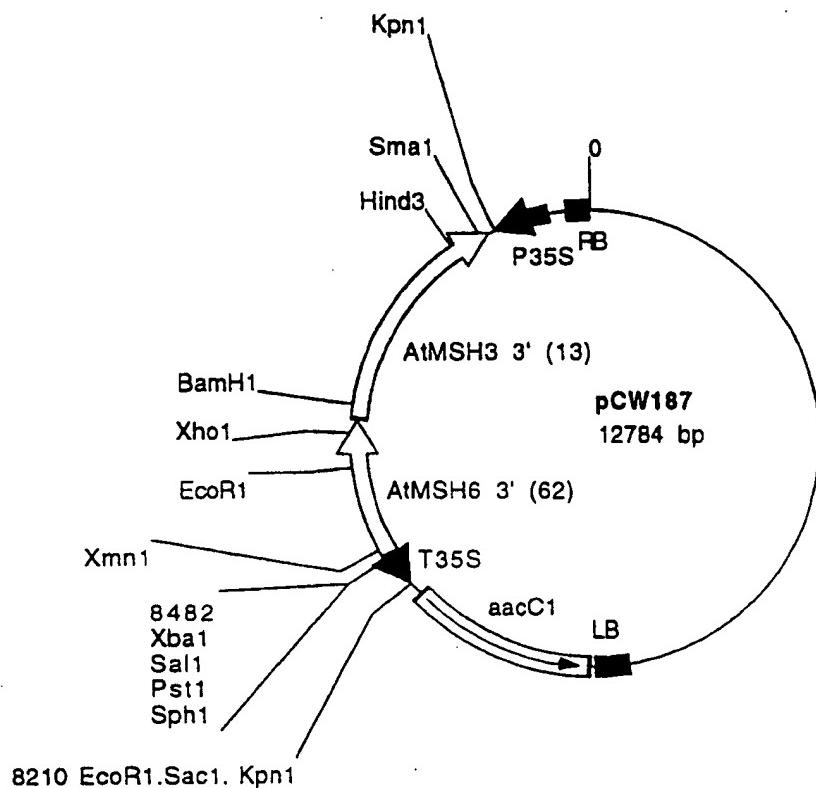
**Figure 13**

**Comments/References:** AtMSH6 (S8) 3' side antisens : 62 Sal1/Sst1/T4 (1379bp)  
into pCW164 BamH1/T4



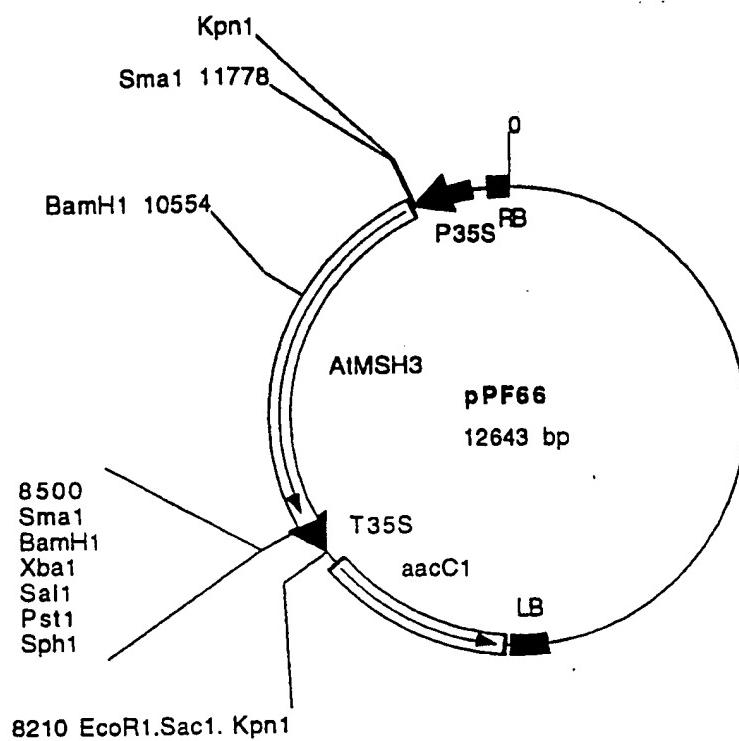
**Figure 14**

**Comments/References:** AtMSH6 3'/AtMSH3 3' antisense : AtMSH6 (S8) 3' side (62=1379bp)  
Sal1/Sst1/T4 into pPF13 (pCW164 AtMSH3 (S5) 3' side (13=2104) antisens)/Sma1. in  
LBA4404



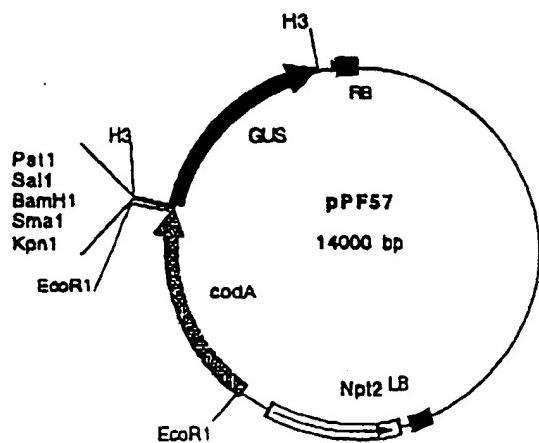
**Figure 15**

**Comments/References:** AtMSH3 3'/AtMSH6 3' antisens (D) : AtMSH3 (S5) 3' side (13=2104bp) Sal1/Sst1/T4 into pPF14 (AtMSH6 (S8) 3'side (62=1379bp) antisense into pCW164)/Sma1. in LBA4404



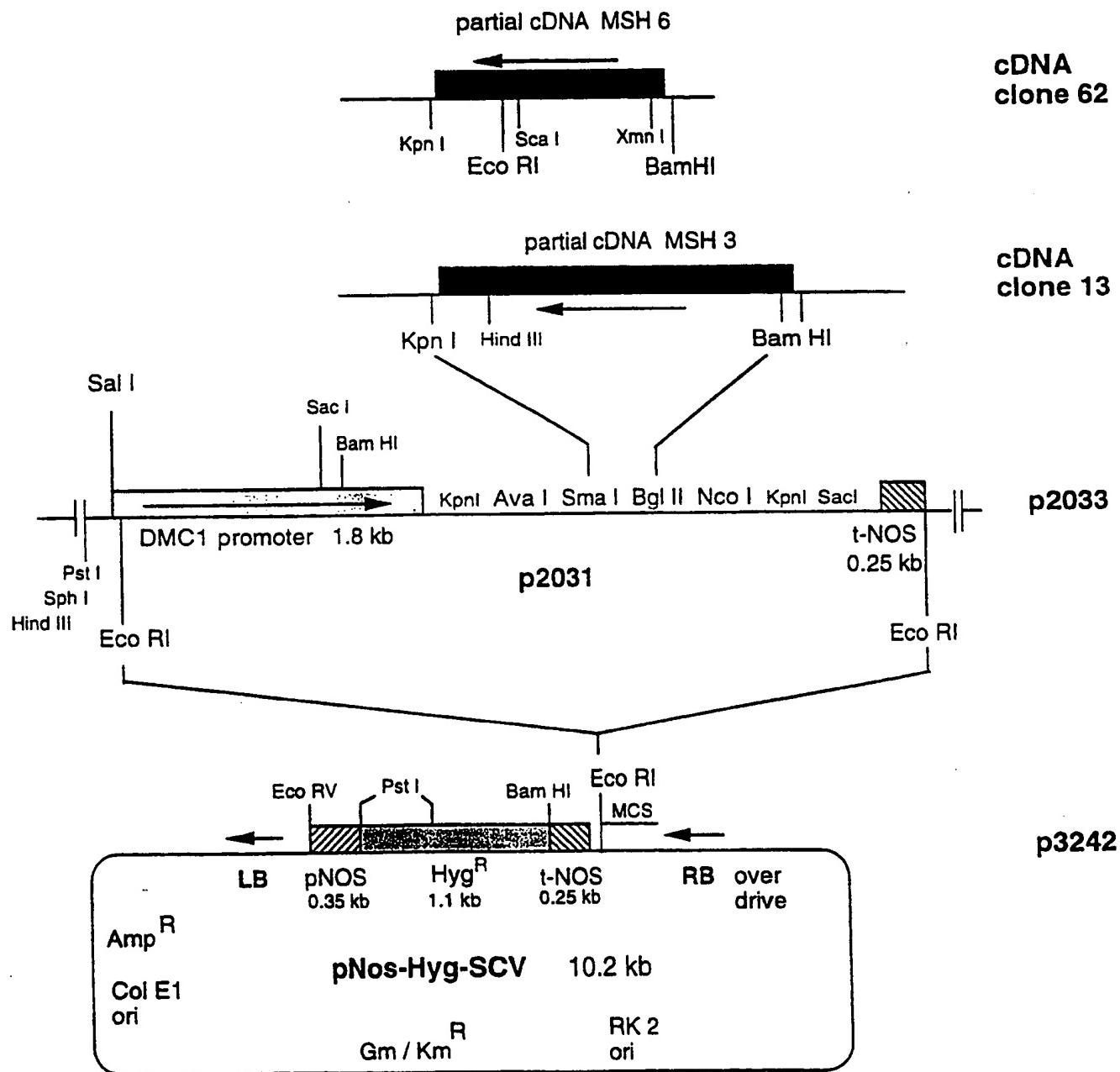
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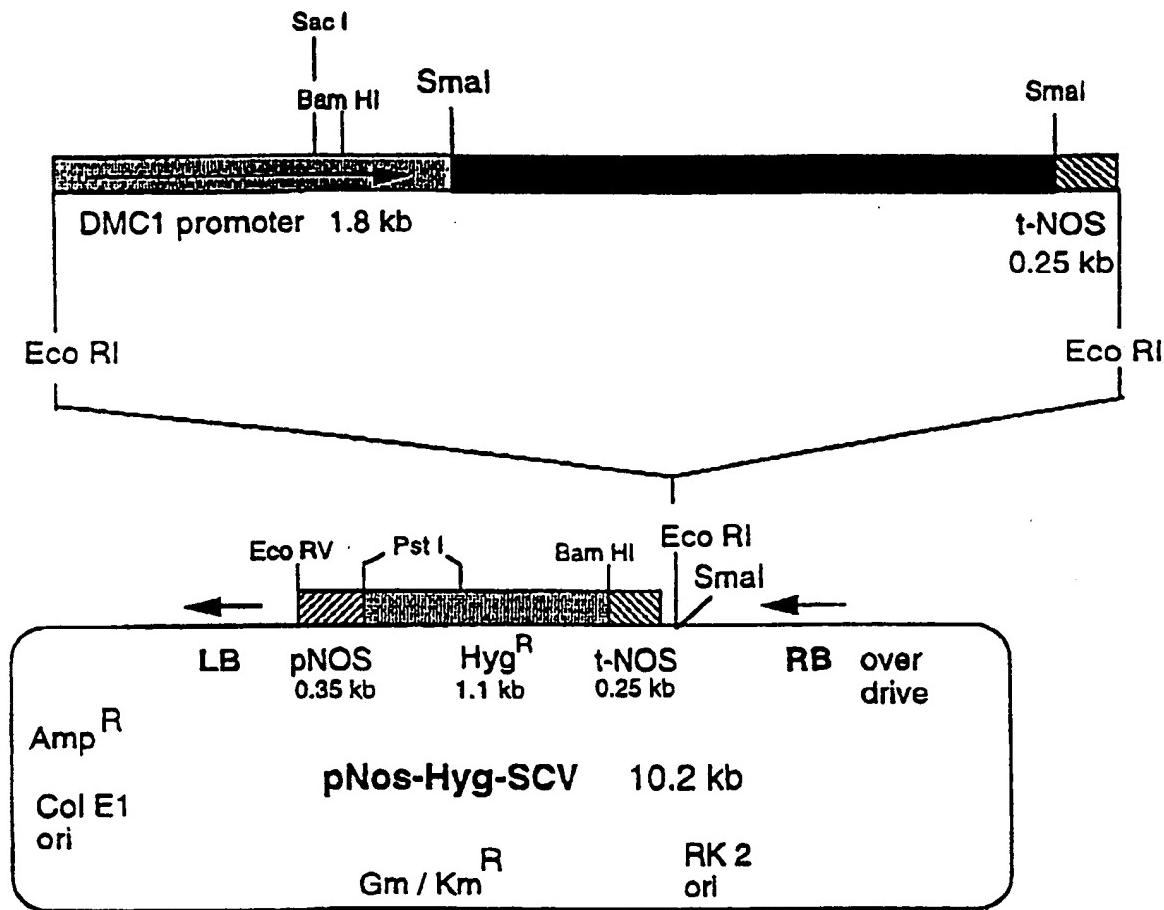
**Comments/References:** AtMSH3 (S8) complete, sense orientation : pPF26 (3342bp)  
Sma1 into pCW164 Sma1



**Figure 17**

**Comments/References:** pPZP111 with coda EcoR1 cassette in EcoR1 site and Hind3 GUS cassette in Hind3 site. KanR. All genes under Promoter/terminator 35S

**Figure 18**

**Figure 19****p3243**

## SEQUENCE LISTING

<110> Rhone-Poulenc Agro; Betzner, Andreas Stefan; Doutriaux, Marie-Pascale; Freyssinet, Georges; Perez, Pascual.

<120> Methods for obtaining plant varieties

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<212> DNA  
<213> Artificial sequence

<220>  
<223> Primer AP2 for PCR using cDNA of *Arabidopsis thaliana* ecotype Columbia containing adapter sequences ligated to both its ends

<400> 5

actcactata gggctcgagc ggc

23

<210> 6  
<211> 30  
<212> DNA  
<213> Artificial sequence

<220>  
<223> MSH3 specific primer S525 for PCR using cDNA of *Arabidopsis thaliana* ecotype Columbia

<400> 6

aggttctgtat tatgtgtgac gctttactta

30

<210> 7  
<211> 29  
<212> DNA  
<213> Artificial sequence

<220>  
<223> MSH3 specific primer S51 for PCR using cDNA of *Arabidopsis thaliana* ecotype Columbia

<400> 7

ggatcgggta ctgggttttg agtgtgagg

29

<210> 8  
<211> 24  
<212> DNA  
<213> Artificial sequence

<220>  
<223> MSH3 specific primer 635 for PCR using cDNA of *Arabidopsis thaliana* ecotype Columbia

<400> 8

gcacgtgctt gatgggtttt tcac 24

<210> 9

<211> 28

<212> DNA

<213> Artificial sequence

<220>

<223> MSH3 specific primer S523 for PCR using cDNA of Arabidopsis thaliana ecotype Columbia

<400> 9

tcagacagta tccagcatgg cagaagta 28

<210> 10

<211> 33

<212> DNA

<213> Artificial sequence

<220>

<223> MSH3 specific primer 1S5 for PCR using cDNA of Arabidopsis thaliana ecotype Columbia

<400> 10

atcccgggat gggcaagcaa aagcagcaga cga 33

<210> 11

<211> 27

<212> DNA

<213> Artificial sequence

<220>

<223> MSH3 specific primer S53 for PCR using cDNA of Arabidopsis thaliana ecotype Columbia

<400> 11

gacaaaagagc gaaatgaggc cccttgg 27

<210> 12

<211> 1250

<212> DNA

<213> Arabidopsis thaliana ecotype Columbia

<223> Clone 52

&lt;400&gt; 12

cccgggatgg gcaagcaaaa gcagcagacg atttctcggt tcttcgtcc caaacccaaa 60  
 tccccgactc acgaaccgaa tccggtagcc gaatcatcaa caccgccacc gaagatatcc 120  
 gccactgtat ctttctctcc ttccaagcgt aagcttctct ccgaccacct cgccgcccgcg 180  
 tcacccaaaa agcctaaact ttctcctcac actcaaaacc cagtacccga tcccaattta 240  
 caccaaagat ttctccagag atttctggaa ccctcgccgg aggaatatgt tcccgaaacg 300  
 tcatcatcga ggaaatacac accatggaa cagcaagtgg tggagctaaa gagcaagtac 360  
 ccagatgtgg ttttgatggg ggaagttggg tacaggtaca gattttcgg agaagacgcg 420  
 gagatcgcag cacgcgtgtt gggtatattac gtcataatgg atcacaattt catgacggcg 480  
 agtgtgccaa catttcgatt gaatttccat gtgagaagac tggtgaatgc aggatacaag 540  
 attggtagtgg tgaaggcagac tgaaactgca gccattaagt cccatggtgc aaaccggacc 600  
 ggccctttt tccggggact gtcggcggtg tataccaaag ccacgcttga agcggctgag 660  
 gatataagtg ggggttggg tggtgaagaa ggttttggg cacagagtaa tttcttgggt 720  
 tgtgttggg atgagagagt taagtcggag acattaggct gtggatttga aatgagttt 780  
 gatgttagag tcgggtttgt tggcggttgaat ttcgacag gtgaagttgt ttatgaagag 840  
 ttcaatgata atttcatgag aagtggatta gaggctgtga tttttagctt gtcaccagct 900  
 gagctgttgc tggccagcc ttttcacaa caaactgaga agttttggg ggcacatgct 960  
 ggacctacct caaacgttgc agtggAACGT gcctcactgg attgtttcag caatggtaat 1020  
 gcagtagatg aggttatttc attatgtgaa aaaatcagcg caggtaactt agaagatgat 1080  
 aaagaaaatga agctggaggc tgctgaaaaa ggaatgtctt gcttgacagt tcatacaatt 1140  
 atgaacatgc cacatctgac tttcaagcc ctgcgcctaa cgtttgcca tctcaaacag 1200  
 tttggatttg aaaggatcct ttaccaaggg gcctcatttc gcttttgc 1250

&lt;210&gt; 13

&lt;211&gt; 34

&lt;212&gt; DNA

&lt;213&gt; Artificial sequence

&lt;220&gt;

&lt;223&gt; MSH3 specific primer 2S5 for PCR using cDNA of Arabidopsis thaliana ecotype Columbia

&lt;400&gt; 13

atccccgggtc aaaatgaaca agttggccttt agtc

34

&lt;210&gt; 14

&lt;211&gt; 27

&lt;212&gt; DNA

&lt;213&gt; Artificial sequence

&lt;220&gt;

<223> MSH3 specific primer S52 for PCR using cDNA of *Arabidopsis thaliana* ecotype Columbia

&lt;400&gt; 14

gccacatctg actgttcaag ccctcg

27

&lt;210&gt; 15

&lt;211&gt; 2110

&lt;212&gt; DNA

<213> *Arabidopsis thaliana* ecotype Columbia

&lt;223&gt; Clone 13

&lt;400&gt; 15

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tgaaaggatc cttaaccaag gggcctcatt tcgctcttg tcaagtaaca cagagatgac	120
tccctcagcc aataactctgc aacagttgga gtttgtaaaa aataattcag atggatcgga	180
atctggccc ttattccata atatgaatca cacacttaca gtatatggtt ccaggcttct	240
tagacactgg gtgactcatc ctctatgcga tagaaatttg atatctgctc ggcttgatgc	300
tgtttcttag atttctgctt gcatggatc tcatagttct tcccaagtcgca gcagttagtt	360
ggttgaagaa ggttctgaga gagcaattgt atcacctgag ttttatctcg tgctctcc	420
agtcttgcaca gctatgtcta gatcatctga tattcaacgt ggaataacaa gaatcttca	480
tcggactgct aaagccacag agttcattgc agttatggaa gctatttac ttgcggggaa	540
gcaaattcag cgcttggca taaagcaaga ctctgaaaatg aggagtagc aatctgcaac	600
tgtgcgatct actctttga gaaaattgat ttctgttatt tcataccctg ttgtgggtga	660
caatgccgga aaacttctct ctgcctaaa taaggaagcg gctgttcgag gtgacttgct	720
cgacatacta attacttcca gcgaccaatt tcctgagctt gctgaagctc gccaaagcgt	780
tttagtcatc agggaaaagc tggattcctc gatagcttca tttcgcaaga agctcgctat	840

tcgaaatttgcgtttc aagtgtcgaa gatcacacat ttgatagagc tgcccggttg 900  
ttccaagggtc cctatgaatt gggtaaaagt aaatagcacc aagaagacta ttcgatatca 960  
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taaggctgcc gttcaagctc ttgctgcact ggactgtttg cactccctt caactctatc 1140  
tagaaaacaag aactatgtcc gtcccgagtt tgtggatgac tgtgaaccag ttgagataaaa 1200  
catacagtct ggtcgatcatc ctgtactgga gactatatta caagataact tcgtccaaaa 1260  
tgacacaatt ttgcatgcag aagggaaata ttgccaaatt atcaccggac ctaacatggg 1320  
aggaaaagagc tgctatatcc gtcaagttgc tttaaattcc ataatggctc aggttggttc 1380  
ctttgtacca gcgtcattcg ccaagctgca cgtgcttgc ggtgtttca ctcggatggg 1440  
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acacataatc agaacctgtt cttctcgatc gcttgttata ttagatgagc ttggaagagg 1560  
caactgcaca cacgacggtg tagccattgc ctatgcaaca ttacagcatc tccttagcaga 1620  
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gagcttttgtt tttaagggttgc ctcagcttgc ccagataacct ccatcatgta tacgtcgagc 1860  
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gggagaacca gaaggacatg aagaaccgag aggccgcagaa gaatcttattt cggctctagg 1980  
tgacttggatc gcagacctga aatttgcctt ctctgaagag gacccttggaa aagcattcga 2040  
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ttgaccgggg 2110

<210> 16  
<211> 29  
<212> DNA  
<213> Artificial sequence

<220>  
<223> MSH3 specific primer S51 for PCR using cDNA of *Arabidopsis*  
thaliana ecotype Columbia

<400> 16

ggatcgggta ctgggttttg agtgtgagg

29

<210> 17  
<211> 30  
<212> DNA  
<213> Artificial sequence

<220>  
<223> MSH3 specific primer S525 for PCR using cDNA of *Arabidopsis thaliana* ecotype Columbia

&lt;400&gt; 17

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30

<210> 18  
<211> 3522  
<212> DNA  
<213> *Arabidopsis thaliana* ecotype Columbia

<220>  
<221> CDS  
<222> (100)....(3342)  
<223> AtMSH3 full-length cDNA and deduced sequence of the encoded polypeptide

&lt;400&gt; 18

cctaagaaaag cgcgcgaaaa ttggcaaccc aagttcgcca tagccacgac cacgacacctc 60

catttctctt aaacggagga gattacgaat aaagcaatt 99

atg ggc aag caa aag cag cag acg att tct cgt ttc ttc gct ccc aaa 147  
Met Gly Lys Gln Lys Gln Gln Thr Ile Ser Arg Phe Phe Ala Pro Lys  
1 5 10 15

ccc aaa tcc ccg act cac gaa ccg aat ccg gta gcc gaa tca tca aca 195  
Pro Lys Ser Pro Thr His Glu Pro Asn Pro Val Ala Glu Ser Ser Thr  
20 25 30

ccg cca ccg aag ata tcc gcc act gta tcc ttc tct cct tcc aag cgt 243  
Pro Pro Pro Lys Ile Ser Ala Thr Val Ser Phe Ser Pro Ser Lys Arg  
35 40 45

aag ctt ctc tcc gac cac ctc gcc gcc gcg tca ccc aaa aag cct aaa 291  
Lys Leu Leu Ser Asp His Leu Ala Ala Ser Pro Lys Lys Pro Lys  
50 55 60

ctt tct cct cac actcaa aac cca gta ccc gat ccc aat tta cac caa 339  
Leu Ser Pro His Thr Gln Asn Pro Val Pro Asp Pro Asn Leu His Gln  
65 70 75 80

aga ttt ctc cag aga ttt ctg gaa ccc tcg ccg gag gaa tat gtt ccc Arg Phe Leu Gln Arg Phe Leu Glu Pro Ser Pro Glu Glu Tyr Val Pro	85	90	95	387	
gaa acg tca tca tcg agg aaa tac aca cca ttg gaa cag caa gtg gtg Glu Thr Ser Ser Ser Arg Lys Tyr Thr Pro Leu Glu Gln Gln Val Val	100	105	110	435	
gag cta aag agc aag tac cca gat gtg ttg atg gtg gaa gtt ggt Glu Leu Lys Ser Lys Tyr Pro Asp Val Val Leu Met Val Glu Val Gly	115	120	125	483	
tac agg tac aga ttc ttc gga gaa gac gcg gag atc gca gca cgc gtg Tyr Arg Tyr Arg Phe Phe Gly Glu Asp Ala Glu Ile Ala Ala Arg Val	130	135	140	531	
ttg ggt att tac gct cat atg gat cac aat ttc atg acg gcg agt gtg Leu Gly Ile Tyr Ala His Met Asp His Asn Phe Met Thr Ala Ser Val	145	150	155	160	579
cca aca ttt cga ttg aat ttc cat gtg aga aga ctg gtg aat gca gga Pro Thr Phe Arg Leu Asn Phe His Val Arg Arg Leu Val Asn Ala Gly	165	170	175		627
tac aag att ggt gta gtg aag cag act gaa act gca gcc att aag tcc Tyr Lys Ile Gly Val Val Lys Gln Thr Glu Thr Ala Ala Ile Lys Ser	180	185	190		675
cat ggt gca aac cgg acc ggc cct ttt ttc cgg gga ctg tcg gcg ttg His Gly Ala Asn Arg Thr Gly Pro Phe Phe Arg Gly Leu Ser Ala Leu	195	200	205		723
tat acc aaa gcc acg ctt gaa gcg gct gag gat ata agt ggt ggt tgt Tyr Thr Lys Ala Thr Leu Glu Ala Ala Glu Asp Ile Ser Gly Gly Cys	210	215	220		771
ggt ggt gaa gaa ggt ttg ggt tca cag agt aat ttc ttg gtt tgt gtt Gly Gly Glu Glu Gly Phe Gly Ser Gln Ser Asn Phe Leu Val Cys Val	225	230	235	240	819
gtg gat gag aga gtt aag tcg gag aca tta ggc tgt ggt att gaa atg Val Asp Glu Arg Val Lys Ser Glu Thr Leu Gly Cys Gly Ile Glu Met	245	250	255		867
agt ttt gat gtt aga gtc ggt gtt ggc gtt gaa att tcg aca ggt Ser Phe Asp Val Arg Val Gly Val Val Gly Val Glu Ile Ser Thr Gly	260	265	270		915
gaa gtt gtt tat gaa gag ttc aat gat aat ttc atg aga agt gga tta Glu Val Val Tyr Glu Glu Phe Asn Asp Asn Phe Met Arg Ser Gly Leu	275	280	285		963

gag gct gtg att ttg agc ttg tca cca gct gag ctg ttg ctt ggc cag Glu Ala Val Ile Leu Ser Leu Ser Pro Ala Glu Leu Leu Leu Gly Gln 290 295 300	1011
cct ctt tca caa caa act gag aag ttt ttg gtg gca cat gct gga cct Pro Leu Ser Gln Gln Thr Glu Lys Phe Leu Val Ala Met Ala Gly Pro 305 310 315 320	1059
acc tca aac gtt cga gtg gaa cgt gcc tca ctg gat tgt ttc agc aat Thr Ser Asn Val Arg Val Glu Arg Ala Ser Leu Asp Cys Phe Ser Asn 325 330 335	1107
ggt aat gca gta gat gag gtt att tca tta tgt gaa aaa atc agc gca Gly Asn Ala Val Asp Glu Val Ile Ser Leu Cys Glu Lys Ile Ser Ala 340 345 350	1155
ggg aac tta gaa gat gat aaa gaa atg aag ctg gag gct gct gaa aaa Gly Asn Leu Glu Asp Asp Lys Glu Met Lys Leu Glu Ala Ala Glu Lys 355 360 365	1203
gga atg tct tgc ttg aca gtt cat aca att atg aac atg cca cat ctg Gly Met Ser Cys Leu Thr Val His Thr Ile Met Asn Met Pro His Leu 370 375 380	1251
act gtt caa gcc ctc gcc cta acg ttt tgc cat ctc aaa cag ttt gga Thr Val Gln Ala Leu Ala Leu Thr Phe Cys His Leu Lys Gln Phe Gly 385 390 395 400	1299
ttt gaa agg atc ctt tac caa ggg gcc tca ttt cgc tct ttg tca agt Phe Glu Arg Ile Leu Tyr Gln Gly Ala Ser Phe Arg Ser Leu Ser Ser 405 410 415	1347
aac aca gag atg act ctc tca gcc aat act ctg caa cag ttg gag gtt Asn Thr Glu Met Thr Leu Ser Ala Asn Thr Leu Gln Gln Leu Glu Val 420 425 430	1395
gtg aaa aat aat tca gat gga tcg gaa tct ggc tcc tta ttc cat aat Val Lys Asn Asn Ser Asp Gly Ser Glu Ser Gly Ser Leu Phe His Asn 435 440 445	1443
atg aat cac aca ctt aca gta tat gct tcc agg ctt ctt aga cac tgg Met Asn His Thr Leu Thr Val Tyr Gly Ser Arg Leu Leu Arg His Trp 450 455 460	1491
gtg act cat cct cta tgc gat aga aat ttg ata tct gct cgg ctt gat Val Thr His Pro Leu Cys Asp Arg Asn Leu Ile Ser Ala Arg Leu Asp 465 470 475 480	1539
gct gtt tct gag att tct gct tgc atg gga tct cat agt tct tcc cag Ala Val Ser Glu Ile Ser Ala Cys Met Gly Ser His Ser Ser Gln 485 490 495	1587

ctc agc agt gag ttg gtt gaa gaa ggt tct gag aga gca att gta tca Leu Ser Ser Glu Leu Val Glu Glu Gly Ser Glu Arg Ala Ile Val Ser 500 505 510	1635
cct gag ttt tat ctc gtg ctc tcc tca gtc ttg aca gct atg tct aga Pro Glu Phe Tyr Leu Val Leu Ser Ser Val Leu Thr Ala Met Ser Arg 515 520 525	1683
tca tct gat att caa cgt gga ata aca aga atc ttt cat cgg act gct Ser Ser Asp Ile Gln Arg Gly Ile Thr Arg Ile Phe His Arg Thr Ala 530 535 540	1731
aaa gcc aca gag ttc att gca gtt atg gaa gct att tta ctt gcg ggg Lys Ala Thr Glu Phe Ile Ala Val Met Glu Ala Ile Leu Leu Ala Gly 545 550 555 560	1779
aag caa att cag cgg ctt ggc ata aag caa gac tct gaa atg agg agt Lys Gln Ile Gln Arg Leu Gly Ile Lys Gln Asp Ser Glu Met Arg Ser 565 570 575	1827
atg caa tct gca act gtg cga tct act ctt ttg aga aaa ttg att tct Met Gln Ser Ala Thr Val Arg Ser Thr Leu Leu Arg Lys Leu Ile Ser 580 585 590	1875
gtt att tca tcc cct gtt gtg gtt gac aat gcc gga aaa ctt ctc tct Val Ile Ser Ser Pro Val Val Asp Asn Ala Gly Lys Leu Leu Ser 595 600 605	1923
gcc cta aat aag gaa gcg gct gtt cga ggt gac ttg ctc gac ata cta Ala Leu Asn Lys Glu Ala Ala Val Arg Gly Asp Leu Leu Asp Ile Leu 610 615 620	1971
atc act tcc agc gac caa ttt cct gag ctt gct gaa gct cgc caa gca Ile Thr Ser Ser Asp Gln Phe Pro Glu Leu Ala Glu Ala Arg Gln Ala 625 630 635 640	2019
gtt tta gtc atc agg gaa aag ctg gat tcc tcg ata gct tca ttt cgc Val Leu Val Ile Arg Glu Lys Leu Asp Ser Ser Ile Ala Ser Phe Arg 645 650 655	2067
aag aag ctc gct att cga aat ttg gaa ttt ctt caa gtg tcg ggg atc Lys Lys Leu Ala Ile Arg Asn Leu Glu Phe Leu Gln Val Ser Gly Ile 660 665 670	2115
aca cat ttg ata gag ctg ccc gtt gat tcc aag gtc cct atg aat tgg Thr His Leu Ile Glu Leu Pro Val Asp Ser Lys Val Pro His Asn Trp 675 680 685	2163
gtg aaa gta aat agc acc aag aag act att cga tat cat ccc cca gaa Val Lys Val Asn Ser Thr Lys Lys Thr Ile Arg Tyr His Pro Pro Glu 690 695 700	2211

ata gta gct ggc ttg gat gag cta gct cta gca act gaa cat ctt gcc Ile Val Ala Gly Leu Asp Glu Leu Ala Leu Ala Thr Glu His Leu Ala 705 710 715 720	2259
att gtg aac cga gct tcg tgg gat agt ttc ctc aag agt ttc agt aga Ile Val Asn Arg Ala Ser Trp Asp Ser Phe Leu Lys Ser Phe Ser Arg 725 730 735	2307
tac tac aca gat ttt aag gct gcc gtt caa gct ctt gct gca ctg gac Tyr Tyr Thr Asp Phe Lys Ala Ala Val Gln Ala Leu Ala Ala Leu Asp 740 745 750	2355
tgt ttg cac tcc ctt tca act cta tct aga aac aag aac tat gtc cgt Cys Leu His Ser Leu Ser Thr Leu Ser Arg Asn Lys Asn Tyr Val Arg 755 760 765	2403
ccc gag ttt gtg gat gac tgt gaa cca gtt gag ata aac ata cag tct Pro Glu Phe Val Asp Asp Cys Glu Pro Val Glu Ile Asn Ile Gln Ser 770 775 780	2451
ggc cgt cat cct gta ctg gag act ata tta caa gat aac ttc gtc cca Gly Arg His Pro Val Leu Glu Thr Ile Leu Gln Asp Asn Phe Val Pro 785 790 795 800	2499
aat gac aca att ttg cat gca gaa ggg gaa tat tgc caa att atc acc Asn Asp Thr Ile Leu His Ala Glu Gly Glu Tyr Cys Gln Ile Ile Thr 805 810 815	2547
gga cct aac atg gga gga aag agc tgc tat atc cgt caa gtt gct tta Gly Pro Asn Met Gly Gly Lys Ser Cys Tyr Ile Arg Gln Val Ala Leu 820 825 830	2595
att tcc ata atg gct cag gtt ggt tcc ttt gta cca gcg tca ttc gcc Ile Ser Ile Met Ala Gln Val Gly Ser Phe Val Pro Ala Ser Phe Ala 835 840 845	2643
aag ctg cac gtg ctt gat ggt gtt ttc act cgg atg ggt gct tca gac Lys Leu His Val Leu Asp Gly Val Phe Thr Arg Met Gly Ala Ser Asp 850 855 860	2691
agt atc cag cat ggc aga agt acc ttt cta gaa gaa tta agt gaa gcg Ser Ile Gln His Gly Arg Ser Thr Phe Leu Glu Leu Ser Glu Ala 865 870 875 880	2739
tca cac ata atc aga acc tgt tct tct cgt tcg ctt gtt ata tta gat Ser His Ile Ile Arg Thr Cys Ser Ser Arg Ser Leu Val Ile Leu Asp 885 890 895	2787
gag ctt gga aga ggc act agc aca cac gac ggt gta gcc att gcc tat Glu Leu Gly Arg Gly Thr Ser Thr His Asp Gly Val Ala Ile Ala Tyr 900 905 910	2835

gca aca tta cag cat ctc cta gca gaa aag aga tgt ttg gtt ctt ttt Ala Thr Leu Gln His Leu Leu Ala Glu Lys Arg Cys Leu Val Leu Phe 915 920 925	2883
gtc acg cat tac cct gaa ata gct gag atc agt aac gga ttc cca ggt Val Thr His Tyr Pro Glu Ile Ala Glu Ile Ser Asn Gly Phe Pro Gly 930 935 940	2931
tct gtt ggg aca tac cat gtc tcg tat ctg aca ttg cag aag gat aaa Ser Val Gly Thr Tyr His Val Ser Tyr Leu Thr Leu Gln Lys Asp Lys 945 950 955 960	2979
ggc agt tat gat cat gat gat gtg acc tac cta tat aag ctt ttg cgt Gly Ser Tyr Asp His Asp Asp Val Thr Tyr Leu Tyr Lys Leu Val Arg 965 970 975	3027
ggt ctt tgc agc agg agc ttt ggt ttt aag gtt gct cag ctt gcc cag Gly Leu Cys Ser Arg Ser Phe Gly Phe Lys Val Ala Gln Leu Ala Gln 980 985 990	3075
ata cct cca tca tgt ata cgt cga gcc att tca atg gct gca aaa ttg Ile Pro Pro Ser Cys Ile Arg Arg Ala Ile Ser Met Ala Ala Lys Leu 995 1000 1005	3123
gaa gct gag gta cgt gca aga gag aga aat aca cgc atg gga gaa cca Glu Ala Glu Val Arg Ala Arg Glu Arg Asn Thr Arg Met Gly Glu Pro 1010 1015 1020	3171
gaa gga cat gaa gaa ccg aga ggc gca gaa gaa tct att tcg gct cta Glu Gly His Glu Glu Pro Arg Gly Ala Glu Glu Ser Ile Ser Ala Leu 1025 1030 1035 1040	3219
ggt gac ttg ttt gca gac ctg aaa ttt gct ctc tct gaa gag gac cct Gly Asp Leu Phe Ala Asp Leu Lys Phe Ala Leu Ser Glu Glu Asp Pro 1045 1050 1055	3267
tgg aaa gca ttc gag ttt tta aag cat gct tgg aag att gct ggc aaa Trp Lys Ala Phe Glu Phe Leu Lys His Ala Trp Lys Ile Ala Gly Lys 1060 1065 1070	3315
atc aga cta aaa cca act tgt tca ttt tgatttaatc ttaacattat Ile Arg Leu Lys Pro Thr Cys Ser Phe 1075 1080	3362
agcaactgca aggtcttgat catctgttag ttgcgtacta acttatgtgt attagtataa caagaaaaga gaatttagaga gatggattct aatccggtgt tgcagtacat cttttctcca ccccataaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa	3422 3482 3522

<210> 19  
<211> 1081  
<212> PRT

<213> *Arabidopsis thaliana* ecotype Columbia  
<223> Polypeptide MSH3

<400> 19

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1 5 10 15

Pro Lys Ser Pro Thr His Glu Pro Asn Pro Val Ala Glu Ser Ser Thr  
20 25 30

Pro Pro Pro Lys Ile Ser Ala Thr Val Ser Phe Ser Pro Ser Lys Arg  
35 40 45

Lys Leu Leu Ser Asp His Leu Ala Ala Ala Ser Pro Lys Lys Pro Lys  
50 55 60

Leu Ser Pro His Thr Gln Asn Pro Val Pro Asp Pro Asn Leu His Gln  
65 70 75 80

Arg Phe Leu Gln Arg Phe Leu Glu Pro Ser Pro Glu Glu Tyr Val Pro  
85 90 95

Glu Thr Ser Ser Ser Arg Lys Tyr Thr Pro Leu Glu Gln Gln Val Val  
100 105 110

Glu Leu Lys Ser Lys Tyr Pro Asp Val Val Leu Met Val Glu Val Gly  
115 120 125

Tyr Arg Tyr Arg Phe Phe Gly Glu Asp Ala Glu Ile Ala Ala Arg Val  
130 135 140

Leu Gly Ile Tyr Ala His Met Asp His Asn Phe Met Thr Ala Ser Val  
145 150 155 160

Pro Thr Phe Arg Leu Asn Phe His Val Arg Arg Leu Val Asn Ala Gly  
165 170 175

Tyr Lys Ile Gly Val Val Lys Gln Thr Glu Thr Ala Ala Ile Lys Ser  
180 185 190

His Gly Ala Asn Arg Thr Gly Pro Phe Phe Arg Gly Leu Ser Ala Leu  
195 200 205

Tyr Thr Lys Ala Thr Leu Glu Ala Ala Glu Asp Ile Ser Gly Gly Cys  
210 215 220

Gly Gly Glu Glu Gly Phe Gly Ser Gln Ser Asn Phe Leu Val Cys Val  
225 230 235 240

Val Asp Glu Arg Val Lys Ser Glu Thr Leu Gly Cys Gly Ile Glu Met  
245 250 255

Ser Phe Asp Val Arg Val Gly Val Val Gly Val Glu Ile Ser Thr Gly  
260 265 270

Glu Val Val Tyr Glu Glu Phe Asn Asp Asn Phe Met Arg Ser Gly Leu  
275 280 285

Glu Ala Val Ile Leu Ser Leu Ser Pro Ala Glu Leu Leu Leu Gly Gln  
290 295 300

Pro Leu Ser Gln Gln Thr Glu Lys Phe Leu Val Ala Met Ala Gly Pro  
305 310 315 320

Thr Ser Asn Val Arg Val Glu Arg Ala Ser Leu Asp Cys Phe Ser Asn  
325 330 335

Gly Asn Ala Val Asp Glu Val Ile Ser Leu Cys Glu Lys Ile Ser Ala  
340 345 350

Gly Asn Leu Glu Asp Asp Lys Glu Met Lys Leu Glu Ala Ala Glu Lys  
355 360 365

Gly Met Ser Cys Leu Thr Val His Thr Ile Met Asn Met Pro His Leu  
370 375 380

Thr Val Gln Ala Leu Ala Leu Thr Phe Cys His Leu Lys Gln Phe Gly  
385 390 395 400

Phe Glu Arg Ile Leu Tyr Gln Gly Ala Ser Phe Arg Ser Leu Ser Ser  
405 410 415

Asn Thr Glu Met Thr Leu Ser Ala Asn Thr Leu Gln Gln Leu Glu Val  
420 425 430

Val Lys Asn Asn Ser Asp Gly Ser Glu Ser Gly Ser Leu Phe His Asn  
435 440 445

Met Asn His Thr Leu Thr Val Tyr Gly Ser Arg Leu Leu Arg His Trp  
450 455 460

Val Thr His Pro Leu Cys Asp Arg Asn Leu Ile Ser Ala Arg Leu Asp  
465 470 475 480

Ala Val Ser Glu Ile Ser Ala Cys Met Gly Ser His Ser Ser Ser Gln  
485 490 495

Leu Ser Ser Glu Leu Val Glu Glu Gly Ser Glu Arg Ala Ile Val Ser  
500 505 510

Pro Glu Phe Tyr Leu Val Leu Ser Ser Val Leu Thr Ala Met Ser Arg  
515 520 525

Ser Ser Asp Ile Gln Arg Gly Ile Thr Arg Ile Phe His Arg Thr Ala  
530 535 540

Lys Ala Thr Glu Phe Ile Ala Val Met Glu Ala Ile Leu Leu Ala Gly  
545 550 555 560

Lys Gln Ile Gln Arg Leu Gly Ile Lys Gln Asp Ser Glu Met Arg Ser  
565 570 575

Met Gln Ser Ala Thr Val Arg Ser Thr Leu Leu Arg Lys Leu Ile Ser  
580 585 590

Val Ile Ser Ser Pro Val Val Asp Asn Ala Gly Lys Leu Leu Ser  
595 600 605

Ala Leu Asn Lys Glu Ala Ala Val Arg Gly Asp Leu Leu Asp Ile Leu  
610 615 620

Ile Thr Ser Ser Asp Gln Phe Pro Glu Leu Ala Glu Ala Arg Gln Ala  
625 630 635 640

Val Leu Val Ile Arg Glu Lys Leu Asp Ser Ser Ile Ala Ser Phe Arg  
645 650 655

Lys Lys Leu Ala Ile Arg Asn Leu Glu Phe Leu Gln Val Ser Gly Ile  
660 665 670

Thr His Leu Ile Glu Leu Pro Val Asp Ser Lys Val Pro His Asn Trp  
675 680 685

Val Lys Val Asn Ser Thr Lys Lys Thr Ile Arg Tyr His Pro Pro Glu  
690 695 700

Ile Val Ala Gly Leu Asp Glu Leu Ala Leu Ala Thr Glu His Leu Ala  
705 710 715 720

Ile Val Asn Arg Ala Ser Trp Asp Ser Phe Leu Lys Ser Phe Ser Arg  
725 730 735

Tyr Tyr Thr Asp Phe Lys Ala Ala Val Gln Ala Leu Ala Ala Leu Asp  
740 745 750

Cys Leu His Ser Leu Ser Thr Leu Ser Arg Asn Lys Asn Tyr Val Arg  
755 760 765

Pro Glu Phe Val Asp Asp Cys Glu Pro Val Glu Ile Asn Ile Gln Ser  
770 775 780

Gly Arg His Pro Val Leu Glu Thr Ile Leu Gln Asp Asn Phe Val Pro  
785 790 795 800

Asn Asp Thr Ile Leu His Ala Glu Gly Glu Tyr Cys Gln Ile Ile Thr  
805 810 815

Gly Pro Asn Met Gly Gly Lys Ser Cys Tyr Ile Arg Gln Val Ala Leu  
820 825 830

Ile Ser Ile Met Ala Gln Val Gly Ser Phe Val Pro Ala Ser Phe Ala  
835 840 845

Lys Leu His Val Leu Asp Gly Val Phe Thr Arg Met Gly Ala Ser Asp  
850 855 860

Ser Ile Gln His Gly Arg Ser Thr Phe Leu Glu Glu Leu Ser Glu Ala  
865 870 875 880

Ser His Ile Ile Arg Thr Cys Ser Ser Arg Ser Leu Val Ile Leu Asp  
885 890 895

Glu Leu Gly Arg Gly Thr Ser Thr His Asp Gly Val Ala Ile Ala Tyr  
900 905 910

Ala Thr Leu Gln His Leu Leu Ala Glu Lys Arg Cys Leu Val Leu Phe  
915 920 925

Val Thr His Tyr Pro Glu Ile Ala Glu Ile Ser Asn Gly Phe Pro Gly  
930 935 940

Ser Val Gly Thr Tyr His Val Ser Tyr Leu Thr Leu Gln Lys Asp Lys  
945 950 955 960

Gly Ser Tyr Asp His Asp Asp Val Thr Tyr Leu Tyr Lys Leu Val Arg  
965 970 975

Gly Leu Cys Ser Arg Ser Phe Gly Phe Lys Val Ala Gln Leu Ala Gln  
980 985 990

Ile Pro Pro Ser Cys Ile Arg Arg Ala Ile Ser Met Ala Ala Lys Leu  
995 1000 1005

Glu Ala Glu Val Arg Ala Arg Glu Arg Asn Thr Arg Met Gly Glu Pro  
 1010 1015 1020

Glu Gly His Glu Glu Pro Arg Gly Ala Glu Glu Ser Ile Ser Ala Leu  
1025 1030 1035 1040

Gly Asp Leu Phe Ala Asp Leu Lys Phe Ala Leu Ser Glu Glu Asp Pro  
1045 1050 1055

Trp Lys Ala Phe Glu Phe Leu Lys His Ala Trp Lys Ile Ala Gly Lys  
1060 1065 1070

Ile Arg Leu Lys Pro Thr Cys Ser Phe  
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24

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<220>  
<223> Primer S81 for PCR using cDNA of *Arabidopsis thaliana* ecotype Columbia

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28

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<211> 30  
<212> DNA  
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24

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<212> DNA

<213> Artificial sequence

<220>

<223> MSH6 specific primer 1S8 for PCR using cDNA of Arabidopsis thaliana ecotype Columbia

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<210> 25

<211> 27

<212> DNA

<213> Artificial sequence

<220>

<223> MSH6 specific primer S83 for PCR using cDNA of Arabidopsis thaliana ecotype Columbia

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27

<210> 26

<211> 1385

<212> DNA

<213> Arabidopsis thaliana ecotype Columbia

<223> Clone 43

<400> 26

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ttaaatgtga aggaagggga tgctaaaggc gacgcttctg tacgtttgc tgtttcgaaa 180

tctgtcgatg aggttagagg aacggatact ccaccggaga aggttccgcg tcgtgtcctg 240

ccgtctggat ttaagccggc tgaatccgcc ggtgatgctt cgtccctgtt ctccaatatt 300

atgcataagt ttgtaaaagt cgatgatcga gattgttctg gagagaggag ccgagaagat 360

gttgttccgc tgaatgattc atctctatgt atgaaggcta atgatgttat tcctcaattt 420

cgttccaata atggtaaaac tcaagaaaaga aaccatgctt ttagttcag tgggagagct 480

gaaccttagat cagtagaaga tataggagta gatggcgatg ttccctggtcc agaaaacacca 540

gggatgcgtc cacgtgcttc tcgcttgaag cgagttctgg aggatgaaat gacttttaag 600

gaggataagg ttccctgtatt ggactctaac aaaaggctga aaatgctcca ggatccggtt 660

tgtggagaga agaaaagaagt aaacgaagga accaaatgg aatggcttga gtcttcgtca	720
atcaggatg ccaatagaag acgtccctgat gatccccttt acgatagaaa gaccttacac	780
ataccacctg atgttttcaa gaaaatgtct gcacacaaa agcaatattt gagggttaag	840
agtgaatata tggacattgt gctttctt aaagtgggaa aattttatga gctgtatgag	900
ctagatgcgg aatttaggtca caaggagctt gactggaaga tgaccatgag tggtgtggaa	960
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aagcatgggg atattttcc ataccaagtt tacaggggtt gtccagaat tgatggccag	1680
acgatgtaa atttttagat atttaacaat agctgtatg gtggcccttc agggaccttg	1740
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acggcaaact cagaaagtat gcaaactact ggccagtatc tccacaaaact tccagactta	1920
gaaagactgc tcggacgcat caagtctagc gttcgatcat cagcctctgt gttgcctgct	1980
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ttcagaagtg gaattgtatct gttgttggct ctacagaagg aatcaaataat gatgagtttgc	2100
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aggaatcaa	tatgatgagt	ttgctttata	aactctgtaa	acttcctata	ttagtaggaa	180
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attatcagaa	ccaagatgtg	acagatgaaa	acgctgaaac	tctcacaata	cttatcgaac	300
tttttatcga	aagagcaact	caatggctg	aggtcattca	caccataagc	tgcctagatg	360
tcctgagatc	tttgcaatc	gcagcaagtc	tctctgtgg	aagcatggcc	aggcctgtta	420
tttttcccgaa	atcagaagct	acagatcaga	atcagaaaac	aaaagggcca	atacttaaaa	480
tccaaggact	atggcatcca	tttgcagttg	cagccgatgg	tcaattgcct	gttccgaatg	540
atataactcct	tggcgaggct	agaagaagca	gtggcagcat	tcatcctcgg	tcattgttac	600
tgacgggacc	aaacatgggc	ggaaaatcaa	ctcttcttcg	tgcaacatgt	ctggccgtta	660
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tcaccaagga	attcgctct	cacccacgtg	tcacctcgaa	acacatggct	tgcgcatca	1020
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taaccgaggg	agcttgcct	gagagctacg	gacttcaagt	ggcactcatg	gctggaatac	1140
caaaccaagt	ggttgaaaca	gcatcaggtg	ctgctcaagc	catgaagaga	tcaattgggg	1200
aaaacttcaa	gtcaagttag	ctaagatctg	agttctcaag	tctgcatgaa	gactggctca	1260
agtcattgggt	gggttatttct	cgagtcgccc	acaacaatgc	ccccattggc	gaagatgact	1320
acgacacttt	gttttgccta	tggcatgaga	tcaaattcctc	ttactgtgtt	cccaaataac	1380

ccggg 1385

<210> 28  
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<212> DNA  
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<210> 29  
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<212> DNA  
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<400> 29

gcgttcgatc atcagcctct gtgttgc 27

<210> 30  
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tggcgccaat ctTccccccc ttTcgaattc tctcagctca aaacatcgTT tctctctcac 120

tctctctcac aattccaaaa a atg cag cgc cag aga tcg att ttg tct ttc 171  
Met Gln Arg Gln Arg Ser Ile Leu Ser Phe  
1 5 10

tcc caa aaa ccc acc gcg gcg act acg aag ggt ttg gtt tcc ggc gat Phe Gln Lys Pro Thr Ala Ala Thr Thr Lys Gly Leu Val Ser Gly Asp	15                    20                    25	219
gct gct agc ggc ggg ggc ggc agc gga gga cca cga ttt aat gtg aag Ala Ala Ser Gly Gly Ser Gly Gly Pro Arg Phe Asn Val Arg	30                    35                    40	267
gaa ggg gat gct aaa ggc gac gct tct gta cgt ttt gct gtt tcg aaa Glu Gly Asp Ala Lys Gly Asp Ala Ser Val Arg Phe Ala Val Ser Lys	45                    50                    55	315
tct gtc gat gag gtt aga gga acg gat act cca ccg gag aag gtt ccg Ser Val Asp Glu Val Arg Gly Thr Asp Thr Pro Pro Glu Lys Val Pro	60                    65                    70	363
cgt cgt gtc ctg ccg tct gga ttt aag ccg gct gaa tcc gcc gst gat Arg Arg Val Leu Pro Ser Gly Phe Lys Pro Ala Glu Ser Ala Gly Asp	75                    80                    85                    90	411
gct tcg tcc ctg ttc tcc aat att atg cat aag ttt gta aaa gtc gat Ala Ser Ser Leu Phe Ser Asn Ile Met His Lys Phe Val Lys Val Asp	95                    100                    105	459
gat cga gat tgt tct gga gag agg agc cga gaa gat gtt gtt ccg ctg Asp Arg Asp Cys Ser Gly Glu Arg Ser Arg Glu Asp Val Val Pro Leu	110                    115                    120	507
aat gat tca tct cta tgt atg aag gct aat gat gtt att cct caa ttt Asn Asp Ser Ser Leu Cys Met Lys Ala Asn Asp Val Ile Pro Gln Phe	125                    130                    135	555
cgt tcc aat aat ggt aaa act caa gaa aga aac cat gct ttt agt ttc Arg Ser Asn Asn Gly Lys Thr Gln Glu Arg Asn His Ala Phe Ser Phe	140                    145                    150	603
agt ggg aga gct gaa ctt aga tca gta gaa gat ata gga gta gat ggc Ser Gly Arg Ala Glu Leu Arg Ser Val Glu Asp Ile Gly Val Asp Gly	155                    160                    165                    170	651
gat gtt cct ggt cca gaa aca cca ggg atg cgt cca cgt gct tct cgc Asp Val Pro Gly Pro Glu Thr Pro Gly Met Arg Pro Arg Ala Ser Arg	175                    180                    185	699
ttg aag cga gtt ctg gag gat gaa atg act ttt aag gag gat aag gtt Leu Lys Arg Val Leu Glu Asp Glu Met Thr Phe Lys Glu Asp Lys Val	190                    195                    200	747
cct gta ttg gac tct aac aaa agg ctg aaa atg ctc cag gat ccg gtt Pro Val Leu Asp Ser Asn Lys Arg Leu Lys Met Leu Gln Asp Pro Val	205                    210                    215	795

tgt gga gag aag aaa gaa gta aac gaa gga acc aaa ttt gaa tgg ctt	220	225	843
Cys Gly Glu Lys Lys Glu Val Asn Glu Gly Thr Lys Phe Glu Trp Leu			
	230		
gag tct tc <sub>1</sub> cga acc agg gat gcc aat aga aga cgt cct gat gat ccc	235	240	891
Glu Ser Ser Arg Ile Arg Asp Ala Asn Arg Arg Arg Pro Asp Asp Pro			
	245	250	
ctt tac gat aga aag acc tta cac ata cca cct gat gtt ttc aag aaa	255	260	939
Leu Tyr Asp Arg Lys Thr Leu His Ile Pro Pro Asp Val Phe Lys Lys			
	265		
atg tct gca tca caa aag caa tat tgg agt gtt aag agt gaa tat atg	270	275	987
Met Ser Ala Ser Gln Lys Gln Tyr Trp Ser Val Lys Ser Glu Tyr Met			
	280		
gac att gtg ctt ttc ttt aaa gtg ggg aaa ttt tat gag ctg tat gag	285	290	1035
Asp Ile Val Leu Phe Phe Lys Val Gly Lys Phe Tyr Glu Leu Tyr Glu			
	295		
cta gat gcg gaa tta ggt cac aag gag ctt gac tgg aag atg acc atg	300	305	1083
Leu Asp Ala Glu Leu Gly His Lys Glu Leu Asp Trp Lys Met Thr Met			
	310		
agt ggt gt <sub>2</sub> gga aaa tgc aga cag gtt atc tct gaa agt ggg ata	315	320	1131
Ser Gly Val Gly Lys Cys Arg Gln Val Gly Ile Ser Glu Ser Gly Ile			
	325	330	
gat gag gca gtg caa aag cta tta gct cgt gga tat aaa gtt gga cga	335	340	1179
Asp Glu Ala Val Gln Lys Leu Leu Ala Arg Gly Tyr Lys Val Gly Arg			
	345		
atc gag cag cta gaa aca tct gac caa gca aaa gcc aga ggt gct aat	350	355	1227
Ile Glu Gin Leu Glu Thr Ser Asp Gln Ala Lys Ala Arg Gly Ala Asn			
	360		
act ata att cca agg aag cta gtt cag gta tta act cca tca aca gca	365	370	1275
Thr Ile Ile Pro Arg Lys Leu Val Gln Val Leu Thr Pro Ser Thr Ala			
	375		
agc gag gga aac atc ggg cct gat gcc gtc cat ctt ctt gct ata aaa	380	385	1323
Ser Glu Gly Asn Ile Gly Pro Asp Ala Val His Leu Leu Ala Ile Lys			
	390		
gag atc aaa atg gag cta caa aag tgt tca act gtg tat gga ttt gct	395	400	1371
Glu Ile Lys Met Glu Leu Gln Lys Cys Ser Thr Val Tyr Gly Phe Ala			
	405	410	
ttt gtt gac tgt gct gcc ttg agg ttt tgg gtt ggg tcc atc agc gat	415	420	1419
Phe Val Asp Cys Ala Ala Leu Arg Phe Trp Val Gly Ser Ile Ser Asp			
	425		

gat gca tca tgt gct gct ctt gga gcg tta ttg atg cag gtt tct cca Asp Ala Ser Cys Ala Ala Leu Gly Ala Leu Leu Met Gln Val Ser Pro 430 435 440	1467
aag gaa gtg tta tat gac agt aaa ggg cta tca aga gaa gca caa aag Lys Glu Val Leu Tyr Asp Ser Lys Gly Leu Ser Arg Glu Ala Gln Lys 445 450 455	1515
gct cta agg aaa tat acg ttg aca ggg tct acg gcg gta cag ttg gct Ala Leu Arg Lys Tyr Thr Leu Thr Gly Ser Thr Ala Val Gln Leu Ala 460 465 470	1563
cca gta cca caa gta atg ggg gat aca gat gct gct gga gtt aga aat Pro Val Pro Gln Val Met Gly Asp Thr Asp Ala Ala Gly Val Arg Asn 475 480 485 490	1611
ata ata gaa tct aac gga tac ttt aaa ggt tct tct gaa tca tgg aac Ile Ile Glu Ser Asn Gly Tyr Phe Lys Gly Ser Ser Glu Ser Trp Asn 495 500 505	1659
tgt gct gtt gat ggt cta aat gaa tgt gat gtt gcc ctt agt gct ctt Cys Ala Val Asp Gly Leu Asn Glu Cys Asp Val Ala Leu Ser Ala Leu 510 515 520	1707
gga gag cta att aat cat ctg tct agg cta aag cta gaa gat gta ctt Gly Glu Leu Ile Asn His Leu Ser Arg Leu Lys Leu Glu Asp Val Leu 525 530 535	1755
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att gat ggc cag acg atg gta aat ctt gag ata ttt aac aat agc tgt Ile Asp Gly Gln Thr Met Val Asn Leu Glu Ile Phe Asn Asn Ser Cys 555 560 565 570	1851
gat ggt ggt cct tca ggg acc ttg tac aaa tat ctt gat aac tgt gtt Asp Gly Gly Pro Ser Gly Thr Leu Tyr Lys Tyr Leu Asp Asn Cys Val 575 580 585	1899
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aaa gat gta gaa agc atc aat aaa cgg ctt gat gta gtt gaa gaa ttc Lys Asp Val Glu Ser Ile Asn Lys Arg Leu Asp Val Val Glu Glu Phe 605 610 615	1995
acg gca aac tca gaa agt atg caa atc act ggc cag tat ctc cac aaa Thr Ala Asn Ser Glu Ser Met Gln Ile Thr Gly Gln Tyr Leu His Lys 620 625 630	2043

ctt cca gac tta gaa aga ctg ctc gga cgc atc aag tct agc gtt cga Leu Pro Asp Leu Glu Arg Leu Leu Gly Arg Ile Lys Ser Ser Val Arg 635 640 645 650	2091
tca tca gcc tct gtg ttg cct gct ctg ggg aaa aaa gtg ctg aaa Ser Ser Ala Ser Val Leu Pro Ala Leu Leu Gly Lys Lys Val Leu Lys 655 660 665	2139
caa cga gtt aaa gca ttt ggg caa att gtg aaa ggg ttc aga agt gga Gln Arg Val Lys Ala Phe Gly Gln Ile Val Lys Gly Phe Arg Ser Gly 670 675 680	2187
att gat ctg ttg ttg gct cta cag aag gaa tca aat atg atg agt ttg Ile Asp Leu Leu Ala Leu Gln Lys Glu Ser Asn Met Met Ser Leu 685 690 695	2235
ctt tat aaa ctc tgt aaa ctt cct ata tta gta gga aaa agc ggg cta Leu Tyr Lys Leu Cys Lys Leu Pro Ile Leu Val Gly Lys Ser Gly Leu 700 705 710	2283
gag tta ttt ctt tct caa ttc gaa gca gcc ata gat agc gac ttt cca Glu Leu Phe Leu Ser Gln Phe Glu Ala Ala Ile Asp Ser Asp Phe Pro 715 720 725 730	2331
aat tat cag aac caa gat gtg aca gat gaa aac gct gaa act ctc aca Asn Tyr Gln Asn Gln Asp Val Thr Asp Glu Asn Ala Glu Thr Leu Thr 735 740 745	2379
ata ctt atc gaa ctt ttt atc gaa aga gca act caa tgg tct gag gtc Ile Leu Ile Glu Leu Phe Ile Glu Arg Ala Thr Gln Trp Ser Glu Val 750 755 760	2427
att cac acc ata agc tgc cta gat gtc ctg aga tct ttt gca atc gca Ile His Thr Ile Ser Cys Leu Asp Val Leu Arg Ser Phe Ala Ile Ala 765 770 775	2475
gca agt ctc tct gct gga agc atg gcc agg cct gtt att ttt ccc gaa Ala Ser Leu Ser Ala Gly Ser Met Ala Arg Pro Val Ile Phe Pro Glu 780 785 790	2523
tca gaa gct aca gat cag aat cag aaa aca aaa ggg cca ata ctt aaa Ser Glu Ala Thr Asp Gln Asn Gln Lys Thr Lys Gly Pro Ile Leu Lys 795 800 805 810	2571
atc caa gga cta tgg cat cca ttt gca gtt gca gcc gat ggt caa ttg Ile Gln Gly Leu Trp His Pro Phe Ala Val Ala Ala Asp Gly Gln Leu 815 820 825	2619
cct gtt ccg aat gat ata ctc ctt ggc gag gct aga aga agc agt ggc Pro Val Pro Asn Asp Ile Leu Leu Gly Glu Ala Arg Arg Ser Ser Gly 830 835 840	2667

agc att cat cct cggtca ttg tta ctg acggaa cca aac atgggc gga Ser Ile His Pro Arg Ser Leu Leu Leu Thr Gly Pro Asn Met Gly Gly	845                    850                    855	2715
aaa tca act ctt ctt cgt gca aca tgt ctggcc gtt atc ttt gcc caa Lys Ser Thr Leu Leu Arg Ala Thr Cys Leu Ala Val Ile Phe Ala Gln	860                    865                    870	2763
ctt ggc tgc tac gtg ccgttgt gag tct tgc gaa atc tcc ctc gtg gat Leu Gly Cys Tyr Val Pro Cys Glu Ser Cys Glu Ile Ser Leu Val Asp	875                    880                    885                    890	2811
act atc ttc aca agg ctt ggc gca tct gat aga atc atg aca gga gag Thr Ile Phe Thr Arg Leu Gly Ala Ser Asp Arg Ile Met Thr Gly Glu	895                    900                    905	2859
agt acc ttt ttgtca gaa tgc act gag aca gcg tca gtt ctt cag aat Ser Thr Phe Leu Val Glu Cys Thr Glu Thr Ala Ser Val Leu Gln Asn	910                    915                    920	2907
gca act cag gat tca cta gta atc ctt gac gaa ctgggc aga gga act Ala Thr Gln Asp Ser Leu Val Ile Leu Asp Glu Leu Gly Arg Gly Thr	925                    930                    935	2955
agt act ttc gat gga tac gcc att gca tac tcg gtt ttt cgt cac ctg Ser Thr Phe Asp Gly Tyr Ala Ile Ala Tyr Ser Val Phe Arg His Leu	940                    945                    950	3003
gtatca gag aaa gtt caa tgt cgg atg ctc ttt gca aca cat tac cac cct Val Glu Lys Val Gln Cys Arg Met Leu Phe Ala Thr His Tyr His Pro	955                    960                    965                    970	3051
ctc acc aag gaa ttc gcgtct cac cca cgt gtc acc tcg aaa cac atg Leu Thr Lys Glu Phe Ala Ser His Pro Arg Val Thr Ser Lys His Met	975                    980                    985	3099
gct tgc gca ttc aaa tca aga tct gat tat caa cca cgt ggt tgt gat Ala Cys Ala Phe Lys Ser Arg Ser Asp Tyr Gln Pro Arg Gly Cys Asp	990                    995                    1000	3147
caa gac cta gtg ttc ttgtac cgt tta acc gag gga gct tgt cct gag Gln Asp Leu Val Phe Leu Tyr Arg Leu Thr Glu Gly Ala Cys Pro Glu	1005                    1010                    1015	3195
agc tac gga ctt caa gtg gca ctc atg gct gga ata cca aac caa gtg Ser Tyr Gly Leu Gln Val Ala Leu Met Ala Gly Ile Pro Asn Gln Val	1020                    1025                    1030	3243
gtt gaa aca gca tca ggt gct gct caa gcc atg aag aga tca att ggg Val Glu Thr Ala Ser Gly Ala Ala Gln Ala Met Lys Arg Ser Ile Gly	1035                    1040                    1045                    1050	3291

gga aac ttc aag tca agt gag cta aga tct gag ttc tca agt ctg cat	3339
Glu Asn Phe Lys Ser Ser Glu Leu Arg Ser Glu Phe Ser Ser Leu His	
1055	1060
	1065
gaa gac tgg ctc aag tca ttg gtt att tct cga gtc gcc cac aac	3387
Glu Asp Trp Leu Lys Ser Leu Val Gly Ile Ser Arg Val Ala His Asn	
1070	1075
	1080
aat gcc ccc att ggc gaa gat gac tac gac act ttg ttt tgc tta tgg	3435
Asn Ala Pro Ile Gly Glu Asp Asp Tyr Asp Thr Leu Phe Cys Leu Trp	
1085	1090
	1095
cat gag atc aaa tcc tct tac tgt gtt ccc aaa taaaatggcta	3478
His Glu Ile Lys Ser Ser Tyr Cys Val Pro Lys	
1100	1105
tgacataaca ctatctgaag ctcgttaagt cttttgcctc tctgatgttt attcctctta	3538
aaaaatgctt atatatcaaa aaattgtttc ctcgattaaa aaaaaaaaaa aaaaaaaaaa	3598
aaaaaaaaa	3606

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Met Gln Arg Gln Arg Ser Ile Leu Ser Phe Phe Gln Lys Pro Thr Ala	
1	5
	10
	15

Ala Thr Thr Lys Gly Leu Val Ser Gly Asp Ala Ala Ser Gly Gly	
20	25
	30

Gly Ser Gly Gly Pro Arg Phe Asn Val Arg Glu Gly Asp Ala Lys Gly	
35	40
	45

Asp Ala Ser Val Arg Phe Ala Val Ser Lys Ser Val Asp Glu Val Arg	
50	55
	60

Gly Thr Asp Thr Pro Pro Glu Lys Val Pro Arg Arg Val Leu Pro Ser	
65	70
	75
	80

Gly Phe Lys Pro Ala Glu Ser Ala Gly Asp Ala Ser Ser Leu Phe Ser	
85	90
	95

Asn Ile Met His Lys Phe Val Lys Val Asp Asp Arg Asp Cys Ser Gly	
100	105
	110

Glu Arg Ser Arg Glu Asp Val Val Pro Leu Asn Asp Ser Ser Leu Cys	
115	120
	125

Met Lys Ala Asn Asp Val Ile Pro Gln Phe Arg Ser Asn Asn Gly Lys  
130 135 140

Thr Gln Glu Arg Asn His Ala Phe Ser Phe Ser Gly Arg Ala Glu Leu  
145 150 155 160

Arg Ser Val Glu Asp Ile Gly Val Asp Gly Asp Val Pro Gly Pro Glu  
165 170 175

Thr Pro Gly Met Arg Pro Arg Ala Ser Arg Leu Lys Arg Val Leu Glu  
180 185 190

Asp Glu Met Thr Phe Lys Glu Asp Lys Val Pro Val Leu Asp Ser Asn  
195 200 205

Lys Arg Leu Lys Met Leu Gln Asp Pro Val Cys Gly Glu Lys Lys Glu  
210 215 220

Val Asn Glu Gly Thr Lys Phe Glu Trp Leu Glu Ser Ser Arg Ile Arg  
225 230 235 240

Asp Ala Asn Arg Arg Pro Asp Asp Pro Leu Tyr Asp Arg Lys Thr  
245 250 255

Leu His Ile Pro Pro Asp Val Phe Lys Lys Met Ser Ala Ser Gln Lys  
260 265 270

Gln Tyr Trp Ser Val Lys Ser Glu Tyr Met Asp Ile Val Leu Phe Phe  
275 280 285

Lys Val Gly Lys Phe Tyr Glu Leu Tyr Glu Leu Asp Ala Glu Leu Gly  
290 295 300

His Lys Glu Leu Asp Trp Lys Met Thr Met Ser Gly Val Gly Lys Cys  
305 310 315 320

Arg Gln Val Gly Ile Ser Glu Ser Gly Ile Asp Glu Ala Val Gln Lys  
325 330 335

Leu Leu Ala Arg Gly Tyr Lys Val Gly Arg Ile Glu Gln Leu Glu Thr  
340 345 350

Ser Asp Gln Ala Lys Ala Arg Gly Ala Asn Thr Ile Ile Pro Arg Lys  
355 360 365

Leu Val Gln Val Leu Thr Pro Ser Thr Ala Ser Glu Gly Asn Ile Gly  
370 375 380

Pro Asp Ala Val His Leu Leu Ala Ile Lys Glu Ile Lys Met Glu Leu  
385 390 395 400

Gln Lys Cys Ser Thr Val Tyr Gly Phe Ala Phe Val Asp Cys Ala Ala  
405 410 415

Leu Arg Phe Trp Val Gly Ser Ile Ser Asp Asp Ala Ser Cys Ala Ala  
420 425 430

Leu Gly Ala Leu Leu Met Gln Val Ser Pro Lys Glu Val Leu Tyr Asp  
435 440 445

Ser Lys Gly Leu Ser Arg Glu Ala Gln Lys Ala Leu Arg Lys Tyr Thr  
450 455 460

Leu Thr Gly Ser Thr Ala Val Gln Leu Ala Pro Val Pro Gln Val Met  
465 470 475 480

Gly Asp Thr Asp Ala Ala Gly Val Arg Asn Ile Ile Glu Ser Asn Gly  
485 490 495

Tyr Phe Lys Gly Ser Ser Glu Ser Trp Asn Cys Ala Val Asp Gly Leu  
500 505 510

Asn Glu Cys Asp Val Ala Leu Ser Ala Leu Gly Glu Leu Ile Asn His  
515 520 525

Leu Ser Arg Leu Lys Leu Glu Asp Val Leu Lys His Gly Asp Ile Phe  
530 535 540

Pro Tyr Gln Val Tyr Arg Gly Cys Leu Arg Ile Asp Gly Gln Thr Met  
545 550 555 560

Val Asn Leu Glu Ile Phe Asn Asn Ser Cys Asp Gly Gly Pro Ser Gly  
565 570 575

Thr Leu Tyr Lys Tyr Leu Asp Asn Cys Val Ser Pro Thr Gly Lys Arg  
580 585 590

Leu Leu Arg Asn Trp Ile Cys His Pro Leu Lys Asp Val Glu Ser Ile  
595 600 605

Asn Lys Arg Leu Asp Val Val Glu Glu Phe Thr Ala Asn Ser Glu Ser  
610 615 620

Met Gln Ile Thr Gly Gln Tyr Leu His Lys Leu Pro Asp Leu Glu Arg  
625 630 635 640

Leu Leu Gly Arg Ile Lys Ser Ser Val Arg Ser Ser Ala Ser Val Leu  
645 650 655

Pro Ala Leu Leu Gly Lys Lys Val Leu Lys Gln Arg Val Lys Ala Phe  
660 665 670

Gly Gln Ile Val Lys Gly Phe Arg Ser Gly Ile Asp Leu Leu Leu Ala  
675 680 685

Leu Gln Lys Glu Ser Asn Met Met Ser Leu Leu Tyr Lys Leu Cys Lys  
690 695 700

Leu Pro Ile Leu Val Gly Lys Ser Gly Leu Glu Leu Phe Leu Ser Gln  
705 710 715 720

Phe Glu Ala Ala Ile Asp Ser Asp Phe Pro Asn Tyr Gln Asn Gln Asp  
725 730 735

Val Thr Asp Glu Asn Ala Glu Thr Leu Thr Ile Leu Ile Glu Leu Phe  
740 745 750

Ile Glu Arg Ala Thr Gln Trp Ser Glu Val Ile His Thr Ile Ser Cys  
755 760 765

Leu Asp Val Leu Arg Ser Phe Ala Ile Ala Ala Ser Leu Ser Ala Gly  
770 775 780

Ser Met Ala Arg Pro Val Ile Phe Pro Glu Ser Glu Ala Thr Asp Gln  
785 790 795 800

Asn Gln Lys Thr Lys Gly Pro Ile Leu Lys Ile Gln Gly Leu Trp His  
805 810 815

Pro Phe Ala Val Ala Ala Asp Gly Gln Leu Pro Val Pro Asn Asp Ile  
820 825 830

Leu Leu Gly Glu Ala Arg Arg Ser Ser Gly Ser Ile His Pro Arg Ser  
835 840 845

Leu Leu Leu Thr Gly Pro Asn Met Gly Gly Lys Ser Thr Leu Leu Arg  
850 855 860

Ala Thr Cys Leu Ala Val Ile Phe Ala Gln Leu Gly Cys Tyr Val Pro  
865 870 875 880

Cys Glu Ser Cys Glu Ile Ser Leu Val Asp Thr Ile Phe Thr Arg Leu  
885 890 895

Gly Ala Ser Asp Arg Ile Met Thr Gly Glu Ser Thr Phe Leu Val Glu  
900 905 910

Cys Thr Glu Thr Ala Ser Val Leu Gln Asn Ala Thr Gln Asp Ser Leu  
915 920 925

Val Ile Leu Asp Glu Leu Gly Arg Gly Thr Ser Thr Phe Asp Gly Tyr  
930 935 940

Ala Ile Ala Tyr Ser Val Phe Arg His Leu Val Glu Lys Val Gln Cys  
945 950 955 960

Arg Met Leu Phe Ala Thr His Tyr His Pro Leu Thr Lys Glu Phe Ala  
965 970 975

Ser His Pro Arg Val Thr Ser Lys His Met Ala Cys Ala Phe Lys Ser  
980 985 990

Arg Ser Asp Tyr Gln Pro Arg Gly Cys Asp Gln Asp Leu Val Phe Leu  
 995 1000 1005

Tyr Arg Leu Thr Glu Gly Ala Cys Pro Glu Ser Tyr Gly Leu Gln Val  
 1010 1015 1020

Ala Leu Met Ala Gly Ile Pro Asn Gln Val Val Glu Thr Ala Ser Gly  
 1025 1030 1035 1040

Ala Ala Gln Ala Met Lys Arg Ser Ile Gly Glu Asn Phe Lys Ser Ser  
 1045 1050 1055

Glu Leu Arg Ser Glu Phe Ser Ser Leu His Glu Asp Trp Leu Lys Ser  
 1060 1065 1070

Leu Val Gly Ile Ser Arg Val Ala His Asn Asn Ala Pro Ile Gly Glu  
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Asp Asp Tyr Asp Thr Leu Phe Cys Leu Trp His Glu Ile Lys Ser Ser  
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 microsatellite

<400> 32

accatgcata gcttaaactt cttg 24

<210> 33  
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 microsatellite

<400> 33

acataaccac aaataggggt gc 22

<210> 34  
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<220>  
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<400> 34

gaagcgatat tgttcgtg 18

<210> 35  
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<212> DNA  
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<220>  
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<400> 35

agattgcgac aacattcc 18

<210> 36  
<211> 31  
<212> DNA  
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<220>  
<223> Forward primer DMCIN-1 for PCR on genomic DNA of Arabidopsis thaliana ssp. Landsberg erecta "Ler"

<400> 36

acgcgtcgac tcagctatga gattactcgt g 31

<210> 37  
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<220>  
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<400> 37

gctctagatt tctcgctcta agactctct 29

<210> 38  
<211> 32  
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<220>  
<223> Forward primer DMCIN-3 for PCR on genomic DNA of *Arabidopsis thaliana* ssp. *Landsberg erecta* "Ler"

<400> 38

gctctagagc ttctcttaag taagtgattg at 32

<210> 39  
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<220>  
<223> Reverse primer DMCIN-4 for PCR on genomic DNA of *Arabidopsis thaliana* ssp. *Landsberg erecta* "Ler"

<400> 39

tccccccgggc tcgagagatc tccatggttt cttcagctct atgaatcc 48

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<220>  
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<400> 40

acgcgtcgac gaattcgcaa gtgggg 26

<210> 41  
<211> 38  
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<220>  
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<400> 41

tccatggaga tctcccggt accgattgc ttcgaggg 38

<210> 42  
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<220>  
<223> Forward primer for PCR amplification of ATEAT1 SSLP marker in Arabidopsis thaliana subspecies

<400> 42

gccactgcgt gaatgatatg 20

<210> 43  
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<220>  
<223> Reverse primer for PCR amplification of ATEAT1 SSLP marker in Arabidopsis thaliana subspecies

<400> 43

cgaacagcca acattaattc cc 22

<210> 44  
<211> 18  
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<220>  
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<400> 44

aaccaaggca cagaagcg 18

<210> 45  
<211> 18  
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<220>  
<223> Reverse primer for PCR amplification of NGA63 SSLP marker in Arabidopsis thaliana subspecies

&lt;400&gt; 45

acccaagtga tcgccacc

18

&lt;210&gt; 46

&lt;211&gt; 21

&lt;212&gt; DNA

&lt;213&gt; Artificial sequence

&lt;220&gt;

<223> Forward primer for PCR amplification of NGA248 SSLP marker in  
Arabidopsis thaliana subspecies

&lt;400&gt; 46

taccgaacca aaacacaaaag g

21

&lt;210&gt; 47

&lt;211&gt; 22

&lt;212&gt; DNA

&lt;213&gt; Artificial sequence

&lt;220&gt;

<223> Reverse primer for PCR amplification of NGA248 SSLP marker in  
Arabidopsis thaliana subspecies

&lt;400&gt; 47

tctgtatctc ggtgaattct cc

22

&lt;210&gt; 48

&lt;211&gt; 22

&lt;212&gt; DNA

&lt;213&gt; Artificial sequence

&lt;220&gt;

<223> Forward primer for PCR amplification of NGA128 SSLP marker in  
Arabidopsis thaliana subspecies

&lt;400&gt; 48

ggtcgttgta tgtcgttaagt cg

22

&lt;210&gt; 49

&lt;211&gt; 22

&lt;212&gt; DNA

&lt;213&gt; Artificial sequence

&lt;220&gt;

<223> Reverse primer for PCR amplification of NGA128 SSLP marker in  
Arabidopsis thaliana subspecies

<400> 49

atcttgaaac cttagggag gg

22

<210> 50

<211> 22

<212> DNA

<213> Artificial sequence

<220>

<223> Forward primer for PCR amplification of NGA280 SSLP marker in  
Arabidopsis thaliana subspecies

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ctgatctcac ggacaatagt gc

22

<210> 51

<211> 20

<212> DNA

<213> Artificial sequence

<220>

<223> Reverse primer for PCR amplification of NGA280 SSLP marker in  
Arabidopsis thaliana subspecies

<400> 51

ggctccataaa aaagtgcacc

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<210> 52

<211> 21

<212> DNA

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<220>

<223> Forward primer for PCR amplification of NGA111 SSLP marker in  
Arabidopsis thaliana subspecies

<400> 52

ctccagttgg aagctaaagg g

21

<210> 53

<211> 21

<212> DNA

<213> Artificial sequence

<220>  
<223> Reverse primer for PCR amplification of NGA111 SSLP marker in  
Arabidopsis thaliana subspecies

<400> 53

tgttttttag gacaaatggc g 21

<210> 54  
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<220>  
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Arabidopsis thaliana subspecies

<400> 54

ccttcacatc caaaaacccac 20

<210> 55  
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Arabidopsis thaliana subspecies

<400> 55

gcacataccc acaaccagaa 20

<210> 56  
<211> 20  
<212> DNA  
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<220>  
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in Arabidopsis thaliana subspecies

<400> 56

cgctacgctt ttccggtaaag 20

<210> 57  
<211> 20  
<212> DNA  
<213> Artificial sequence  
  
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<223> Reverse primer for PCR amplification of NGA1126 SSLP marker in *Arabidopsis thaliana* subspecies  
  
<400> 57

gcacagtc ca agtcacaacc 20

<210> 58  
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<223> Forward primer for PCR amplification of NGA361 SSLP marker in *Arabidopsis thaliana* subspecies  
  
<400> 58

aaagagatga gaatttggac 20

<210> 59  
<211> 23  
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<400> 59

acatatcaat atattaaagt agc 23

<210> 60  
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<400> 60

tcgtctactg cactgccg

18

<210> 61  
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<400> 61

gaggacatgt ataggagcct cg

22

<210> 62  
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<212> DNA  
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<223> Forward primer for PCR amplification of AthBIO2 SSLP marker in Arabidopsis thaliana subspecies

<400> 62

tgacctcc tc ttccatggag

20

<210> 63  
<211> 22  
<212> DNA  
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<220>  
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<400> 63

ttaacagaaa cccaaagtt tc

22

<210> 64  
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<400> 64

aggcaaatgt ccatttcatt g

21

<210> 65

<211> 20

<212> DNA

<213> Artificial sequence

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<223> Reverse primer for PCR amplification of AthUBIQUE SSLP marker in *Arabidopsis thaliana* subspecies

<400> 65

acgacatggc agatttctcc

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<210> 66

<211> 21

<212> DNA

<213> Artificial sequence

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<223> Forward primer for PCR amplification of NGA172 SSLP marker in *Arabidopsis thaliana* subspecies

<400> 66

agctgcttcc ttatagcgtc c

21

<210> 67

<211> 19

<212> DNA

<213> Artificial sequence

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<223> Reverse primer for PCR amplification of NGA172 SSLP marker in *Arabidopsis thaliana* subspecies

<400> 67

catccgaatg ccattgttc

19

<210> 68

<211> 21

<212> DNA

<213> Artificial sequence

<220>

42

<223> Forward primer for PCR amplification of NGA126 SSLP marker in  
Arabidopsis thaliana subspecies

<400> 63

aaaaaaaacgc tactttcgtg g

21

<210> 69

<211> 22

<212> DNA

<213> Artificial sequence

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<223> Reverse primer for PCR amplification of NGA126 SSLP marker in  
Arabidopsis thaliana subspecies

<400> 69

caagagcaat atcaagagca gc

22

<210> 70

<211> 20

<212> DNA

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<220>

<223> Forward primer for PCR amplification of NGA162 SSLP marker in  
Arabidopsis thaliana subspecies

<400> 70

catgcaattt gcatctgagg

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<210> 71

<211> 22

<212> DNA

<213> Artificial sequence

<220>

<223> Reverse primer for PCR amplification of NGA162 SSLP marker in  
Arabidopsis thaliana subspecies

<400> 71

ctctgtcact cttttcctct gg

22

<210> 72

<211> 21

<212> DNA

<213> Artificial sequence

<220>  
<223> Forward primer for PCR amplification of NGA6 SSLP marker in  
Arabidopsis thaliana subspecies

<400> 72

tggatttctt cctctttca c 21

<210> 73  
<211> 21  
<212> DNA  
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<220>  
<223> Reverse primer for PCR amplification of NGA6 SSLP marker in  
Arabidopsis thaliana subspecies

<400> 73

atggagaagc ttacactgat c 21

<210> 74  
<211> 20  
<212> DNA  
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<220>  
<223> Forward primer for PCR amplification of NGA12 SSLP marker in  
Arabidopsis thaliana subspecies

<400> 74

aatgttgtcc tcccccttc 20

<210> 75  
<211> 22  
<212> DNA  
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<220>  
<223> Reverse primer for PCR amplification of NGA12 SSLP marker in  
Arabidopsis thaliana subspecies

<400> 75

tgatgctctc tgaaaacaaga gc 22

<210> 76  
<211> 21  
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<223> Forward primer for PCR amplification of NGA8 SSLP marker in Arabidopsis thaliana subspecies

<400> 76

gagggcaaat ctttatccg g 21

<210> 77  
<211> 22  
<212> DNA  
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<400> 77

tggcttcgt ttataaacat cc 22

<210> 78  
<211> 21  
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<223> Forward primer for PCR amplification of NGA1107 SSLP marker in Arabidopsis thaliana subspecies

<400> 78

gcgaaaaaac aaaaaaatcc a 21

<210> 79  
<211> 21  
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<400> 79

cgacgaatcg acagaattag g

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<210> 80

<211> 21

<212> DNA

<213> Artificial sequence

<220>

<223> Forward primer for PCR amplification of NGA225 SSLP marker in  
Arabidopsis thaliana subspecies

<400> 80

gaaatccaaa tcccagagag g

21

<210> 81

<211> 22

<212> DNA

<213> Artificial sequence

<220>

<223> Reverse primer for PCR amplification of NGA225 SSLP marker in  
Arabidopsis thaliana subspecies

<400> 81

tctccccact agttttgtgt cc

22

<210> 82

<211> 19

<212> DNA

<213> Artificial sequence

<220>

<223> Forward primer for PCR amplification of NGA249 SSLP marker in  
Arabidopsis thaliana subspecies

<400> 82

taccgtcaat ttcatcgcc

19

<210> 83

<211> 22

<212> DNA

<213> Artificial sequence

<220>

<223> Reverse primer for PCR amplification of NGA249 SSLP marker in  
Arabidopsis thaliana subspecies

<400> 83

ggatccctaa ctgtaaaatc cc 22

<210> 84

<211> 22

<212> DNA

<213> Artificial sequence

<220>

<223> Forward primer for PCR amplification of CA72 SSLP marker in  
Arabidopsis thaliana subspecies

<400> 84

aatcccagta accaaacaca ca

22

<210> 85

<211> 20

<212> DNA

<213> Artificial sequence

<220>

<223> Reverse primer for PCR amplification of CA72 SSLP marker in  
Arabidopsis thaliana subspecies

<400> 85

cccagtctaa ccacgaccac

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<210> 86

<211> 20

<212> DNA

<213> Artificial sequence

<220>

<223> Forward primer for PCR amplification of NGA151 SSLP marker in  
Arabidopsis thaliana subspecies

<400> 86

gttttggaa gtttgctgg

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<210> 87

<211> 24

<212> DNA

<213> Artificial sequence

<220>

<223> Reverse primer for PCR amplification of NGA151 SSLP marker in  
Arabidopsis thaliana subspecies

<400> 87

cagtctaaaa gcgagagttat gatg

24

<210> 88

<211> 22

<212> DNA

<213> Artificial sequence

<220>

<223> Forward primer for PCR amplification of NGA106 SSLP marker in  
Arabidopsis thaliana subspecies

<400> 88

gttatggagt ttcttagggca cg

22

<210> 89

<211> 20

<212> DNA

<213> Artificial sequence

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<223> Reverse primer for PCR amplification of NGA106 SSLP marker in  
Arabidopsis thaliana subspecies

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<210> 90

<211> 20

<212> DNA

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<223> Forward primer for PCR amplification of NGA139 SSLP marker in  
Arabidopsis thaliana subspecies

<400> 90

agagctacca gatccgatgg

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<210> 91

<211> 21

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Arabidopsis thaliana subspecies

<400> 91

ggtttcgttt cactatccag g 21

<210> 92  
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## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification <sup>6</sup> : <b>C12N 15/82, 15/29, C07K 14/415, C12N 15/10, 5/04, 5/14, A01H 5/00</b>		A3	(11) International Publication Number: <b>WO 99/19492</b> (43) International Publication Date: <b>22 April 1999 (22.04.99)</b>
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(74) Agent: GENIN, Patrick; Rhône-Poulenc Agro, DPI, 14/20, rue Pierre Baizet, F-69009 Lyon (FR).		(88) Date of publication of the international search report:	24 June 1999 (24.06.99)

(54) Title: METHODS FOR OBTAINING PLANT VARIETIES

## (57) Abstract

An isolated and purified DNA molecule comprising a polynucleotide sequence encoding a polypeptide functionally involved in the DNA mismatch repair system of a plant.

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DK	Denmark	LR	Liberia	SG	Singapore		
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# INTERNATIONAL SEARCH REPORT

Int. onal Application No  
PCT/EP 98/06977

<b>A. CLASSIFICATION OF SUBJECT MATTER</b>					
IPC 6	C12N15/82	C12N15/29	C07K14/415	C12N15/10	C12N5/04
	C12N5/14	A01H5/00			

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)  
IPC 6 C12N A01H

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 95 15381 A (CHAPELLE ALBERT DE ;UNIV JOHNS HOPKINS (US)) 8 June 1995 see the whole document ---	1-5, 33-35
A	WO 97 37011 A (SETRATECH S A R L ;BORTS RHONA HARRIET (GB); LOUIS EDWARD JOHN (GB)) 9 October 1997 see abstract see the whole document ---	1-35
A	WO 90 07576 A (SETRATECH) 12 July 1990 see the whole document and specially page 5, line 16-31, examples 3-4 ---	1,7-27, 31,32 -/-

Further documents are listed in the continuation of box C.

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Mateo Rosell, A.M.

## INTERNATIONAL SEARCH REPORT

International Application No  
PCT/EP 98/06977

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 97 01634 A (ANGELETTI P IST RICERCHE BIO ;JIRICNY JOSEF (IT); PALOMBO FABIO () 16 January 1997 see page 1, line 18 - page 2, line 28 see page 58 ---	1,2
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